CHEMICAL CONSTITUENTS FROM THE ROOTS OF *Cratoxylum cochinchinense*

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องค์ประกอบทางเคมีจากรากติ้วเกลี้ยง *Cratoxylum cochinchinense*

นายพีรวัฒน์ เนตรสง่า

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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By	Mr. Peeravat Natrsanga
Field of Study	Chemistry
Thesis Advisor	Associate Professor SANTI TIP-PYANG, Ph.D.

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พีรวัฒน์ เนตรสง่า : องค์ประกอบทางเคมีจากรากติ้วเกลี้ยง *Cratoxylum cochinchinense*. (CHEMICAL CONSTITUENTS FROM THE ROOTS OF *Cratoxylum cochinchinense*) อ.ที่ปรึกษาหลัก : รศ. ดร.สันติ ทิพยางค์

การศึกษาองค์ประกอบทางเคมีจากสิ่งสกัดไดคลอไรมีเทนของรากต้นติ้วเกลี้ยง สามารถแยกสารในกลุ่ม Xanthone ได้ทั้งหมด 20 สาร ประกอบด้วย สารชนิดใหม่ 4 สาร ได้แก่ cratochinone A-D (1-4) และสารที่เคยมีการรายงานโครงสร้างก่อนหน้านี้ 16 สาร โดยโครงสร้าง Xanthone ที่แยกได้ทั้งหมด 20 สาร ได้พิสูจน์ทราบเอกลักษณ์โครงสร้างทางเคมีด้วยวิธีทางสเปกโทรสโกปี (1D และ 2D NMR) และ เปรียบเทียบข้อมูลจากสารที่เคยมีการรายงานก่อนหน้านี้ จากนั้นทำการทดสอบความเป็นพิษต่อเซลล์มะเร็งชนิด KB, HeLa S-3, HT-29, MCF-7 และ Hep G2 ของสารบริสุทธิ์ทั้งหมดที่แยกได้ พบว่าสารส่วนใหญ่มีฤทธิ์ในการยับยั้งเซลล์มะเร็งในระดับปานกลางไปจนถึงไม่มีฤทธิ์ ยกเว้นสารหมายเลข 2, 7 และ 9 มีความเป็นพิษต่อเซลล์ในระดับดีกับเซลล์ KB, HeLa S-3, HT-29, MCF-7 และ Hep G2 โดยมีค่า IC_{50} อยู่ในช่วง 0.91–9.93 μM ในขณะที่สารหมายเลข 12 มีความเป็นพิษต่อเซลล์ KB, HeLa S-3, and HT-29 โดยมีค่า IC_{50} เท่ากับ 7.39, 6.07, and 8.11 μM ตามลำดับ และสุดท้ายสารหมายเลข 14 มีความเป็นพิษต่อเซลล์เฉพาะ KB and HeLa S-3 โดยมีค่า IC_{50} เท่ากับ 7.28 and 9.84 μM.

สาขาวิชา เคมี ลายมือชื่อนิสิต .....

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Peeravat Natrsanga : CHEMICAL CONSTITUENTS FROM THE ROOTS OF *Cratoxylum cochinchinense*. Advisor: Assoc. Prof. SANTI TIP-PYANG, Ph.D.

The phytochemical investigation of the CH_2Cl_2 extract from the roots of *Cratoxylum cochinchinense* led to the isolation of four new xanthone derivatives, namely cratochinone A-D (1-4), along with sixteen known xanthones (5-20). Their structures were characterized by spectroscopic methods, especially spectroscopic data (1D and 2D NMR) as well as comparison with those reported in the literature for known xanthones. Moreover, all isolated compounds were evaluated for their cytotoxicity against five human cancer cell lines (KB, HeLa S-3, HT-29, MCF-7 and Hep G2 cell lines). Compounds 2, 7, and 9 showed significant cytotoxic effects against all cell lines with IC_{50} values in the range of 0.91–9.93 μM, while 12 exhibited cytotoxicity against the KB, HeLa S-3, and HT-29 cells with IC_{50} values of 7.39, 6.07, and 8.11 μM, respectively. Compound 14 exhibited cytotoxicity against both KB and HeLa S-3 cells with IC_{50} values of 7.28 and 9.84 μM.

Field of Study: Chemistry Student's Signature .....

Academic Year: 2019 Advisor's Signature .....

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Peeravat Natrsanga

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IC_{50}	The molar concentration of an antagonist that reduces the response to an agonist by 50%
kg	kilogram
µg	microgram
µM	micromolar
L	liter
mL	milliliter
mmol	millimole
h	hour(s)
U	unit
m/z	mass per charge number of ions (Mass Spectroscopy)
δ	chemical shift (NMR)
δ_H	chemical shift of proton (NMR)
δ_C	chemical shift of carbon (NMR)
J	coupling constant (NMR)
s	singlet (NMR)
d	doublet (NMR)
dd	doublet of doublet (NMR)
t	triplet (NMR)
brs	broad singlet (NMR)

Hz	hertz
MHz	megahertz
DMSO-d_6	deuterated dimethyl sulfoxide
$CDCl_3$	deuterated chloroform
HRESIMS	high resolution electrospray ionization mass spectroscopy
^{1}H-NMR	proton nuclear magnetic resonance
^{13}C-NMR	carbon-13 nuclear magnetic resonance
1D-NMR	one dimensional nuclear magnetic resonance
2D-NMR	two-dimensional nuclear magnetic resonance
COSY	correlation spetroscopy
HSQC	heteronuclear single quantum correlation
HMBC	heteronuclear multiple bond correlation
calcd.	calculated
TLC	thin layer chromatography
MEM	minimum Essential Media
HEX	hexane
DCM	dichloromethane
DMSO	dimethyl sulfoxide
EtOH	ethanol
EtOAc	ethyl acetate
M	methanol

D	dichloromethane
E	ethyl acetate
H	hexane

CHAPTER I
INTRODUCTION

In the present, plants are being used to treat several health concerns and conditions, including arthritis, migraines, fatigue, skin infections, wounds, burns, gastrointestinal and even cancer proving that is true that food is medicine. These herbs are less expensive and have little side effects more than conventional medications, which is why so many people are focus on the investigation of the efficacy of plant in the traditional medicine.

Plants are rich in a variety of compounds. Many are secondary metabolites and include aromatic substances, most of which are phenols or their oxygen-substituted derivatives such as tannins [1, 2]. Many of these compounds have antioxidant properties. About 200 years ago, the use of natural products as medicines has been described throughout history in the form of traditional medicines, remedies, potions and oils with many of these bioactive natural products still being unidentified. The dominant source of knowledge of natural product uses from medicinal plants is a result of man experimenting by trial and error for hundreds of centuries through palatability trials or untimely deaths, searching for available foods for the treatment of diseases [3, 4]. One example involves the plant genus Salvia which grows throughout the southwestern region of the United States as well as northwestern Mexico and which was used by Indian tribes of southern California as an aid in childbirth [3]. Male newborn babies were “cooked” in the hot Salvia ashes as it was believed that these babies consistently grew to be the strongest and healthiest members of their respective tribes and are claimed to have been immune from all respiratory ailments for life [3]. Moreover, traditional medicinal practices have formed the basis of most of the early medicines followed by subsequent clinical, pharmacological and chemical studies [5]. Probably the most famous and well-known example to date would be the synthesis of the anti-inflammatory agent,

acetylsalicyclic acid (**1**) (aspirin) derived from the natural product, salicin (**2**) isolated from the bark of the willow tree *Salix alba* L. [6]. Investigation of *Papaver somniferum* L. (opium poppy) resulted in the isolation of several alkaloids including morphine (**3**), a commercially important drug, first reported in 1803 (**Figure 1.1**). It was in the 1870s that crude morphine derived from the plant *P. somniferum*, was boiled in acetic anhydride to yield diacetylmorphine (heroin) and found to be readily converted to codeine (painkiller). Historically, it is documented that the Sumerians and Ancient Greeks used poppy extracts medicinally, whilst the Arabs described opium to be addictive [6]. *Digitalis purpurea* L. (foxglove) had been traced back to Europe in the 10th century but it was not until the 1700s that the active constituent digitoxin (**4**), a cardiotonic glycoside was found to enhance cardiac conduction, thereby improving the strength of cardiac contractibility. Digitoxin (**4**) and its analogues have long been used in the management of congestive heart failure and have possible long term detrimental effects and are being replaced by other medicines in the treatment of "heart deficiency" [6]. The anti-malarial drug quinine (**5**) approved by the United States FDA in 2004, isolated from the bark of *Cinchona succirubra* Pav. ex Klotsch, had been used for centuries for the treatment of malaria, fever, indigestion, mouth and throat diseases and cancer. Formal use of the bark to treat malaria was established in the mid-1800s when the British began the worldwide cultivation of the plant [6]. Pilocarpine (**6**) found in *Pilocarpus jaborandi* (Rutaceae) is an L-histidine-derived alkaloid, which has been used as a clinical drug in the treatment of chronic open-angle glaucoma and acute angle-closure glaucoma for over 100 years. Also, more than 60% of cancer therapeutics on the market or in testing are based on natural products. Of 177 drugs approved worldwide for treatment of cancer, more than 70% are based on natural products or mimetics, many of which are improved with combinatorial chemistry. Cancer therapeutics from plants include paclitaxel, isolated from the Pacific yew tree; camptothecin, derived from the Chinese "happy tree" *Camptotheca acuminata* and used to prepare

irinotecan and topotecan; and combretastatin, derived from the South African bush willow [7]. It is also estimated that about 25% of the drugs prescribed worldwide are derived from plants, and 121 such active compounds are in use [8]. Between 2005 and 2007, 13 drugs derived from natural products were approved in the United States. More than 100 natural product-based drugs are in clinical studies [9], and of the total 252 drugs in the World Health Organization's (WHO) essential medicine list, 11% are exclusively of plant origin [8].

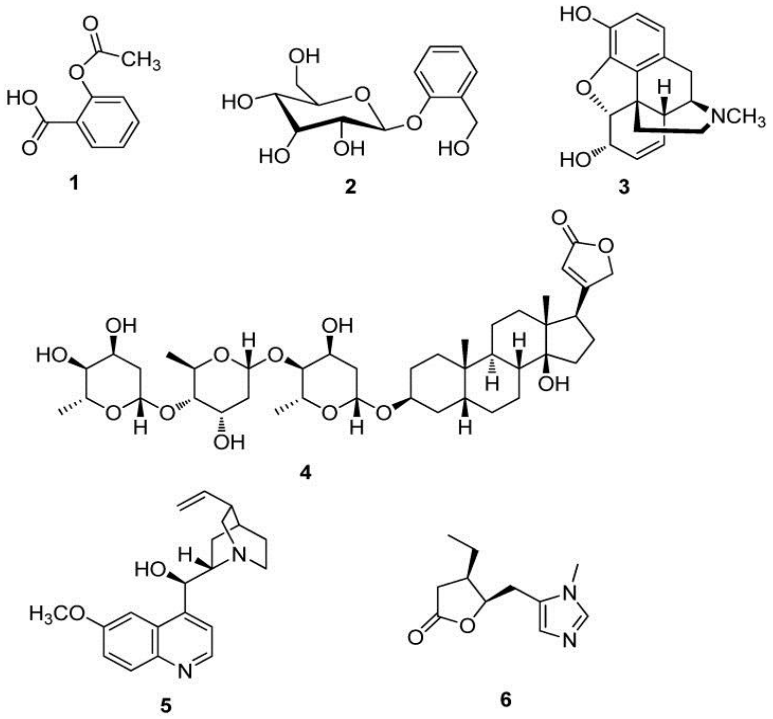


Figure 1.1 Natural products derived from plants

1.1 Xanthones: biosynthesis pathway and biological activities

Xanthones (IUPAC name 9H-Xanthen-9-one) are a kind of phenolic acid with a three-ring skeleton, which are one of the biggest classes of compounds in natural product chemistry. A number of xanthones have been isolated from natural sources of higher plants, fungi, ferns, and lichens. These constituents display a vast range of bioactivities, including anticancer, antioxidative, antimicrobial, antidiabetic, antiviral and anti-inflammatory effects, and so on.

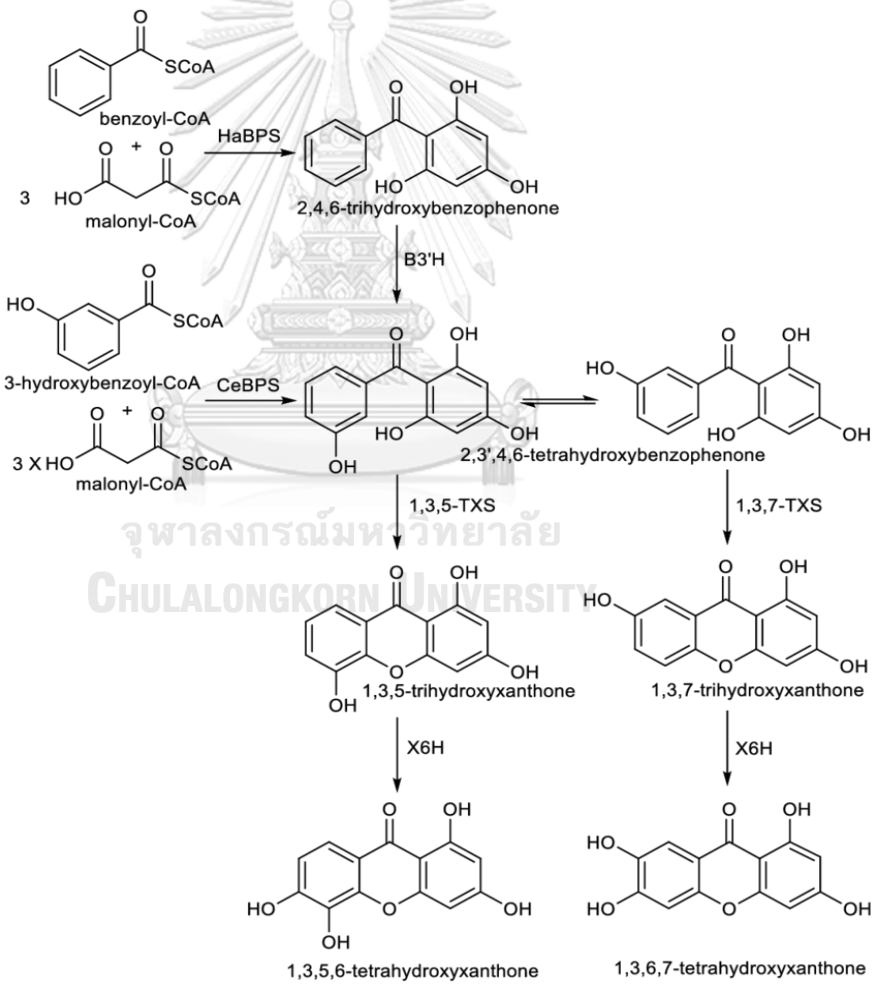


Figure 1.2 Overview of major xanthones biosynthesis pathway in plants

Xanthones are mainly isolated from herbal medicines. Between 1988 and 2016, 168 species of herbal medicinal plant belonging to 58 genera, and 24 families were found to be enriched in xanthones. Among them, the Calophyllum, Cratoxylum, Cuddrania, Garcinia, Gentiana, Hypericum and Swertia genera are the plant resource with the most development prospect [10].

Moreover, the genus *Cratoxylum*, belonging to the family Hypericaceae, which is widely distributed in Southeast Asia. This genus has been shown to possess various pharmacological activities including antioxidants, antimalarial, antibacterial, anti-HIV, and cytotoxic activities. This genus has an abundant source of secondary metabolites, especially xanthones, flavonoids, tocotrienols, and triterpenoids [11-13].

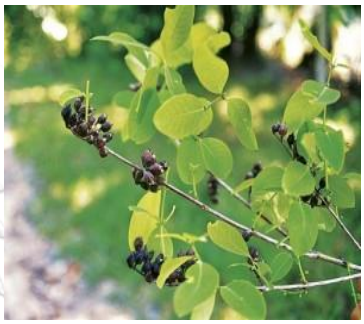
1.2 Botanical aspect and distribution of *Cratoxylum cochinchinense*

Cratoxylum cochinchinense (Lour.) Blume (**Figure 1.3**) is a large to shrubby tree belonging to the family *Hypericaceae*, which is widely distributed in Southeast Asia. This tree is a large to shrub and tall 3-8 m with slender branches. Its bark is gray, vrack and flaking in small irregular pieces. Its leaves have an elliptical shape with 2-4 cm width and 4-10 cm long. Its stalks have long lower than 3 cm. Their flowers have crimson or dark red, which have five sepals and petals at end of twig and in axils of mature leaves. Its fruit is elliptical shape, which 0.8-1.2 cm width, about 2/3 of fruit covered by the persistent sepals [14].

whole plant

stem

Flower



fruit

Figure 1.3 The whole plant, stem, flower and fruit of *Cratoxylum cochinchinense*

Family : *Hypericaceae*

Genus : *Cratoxylum*

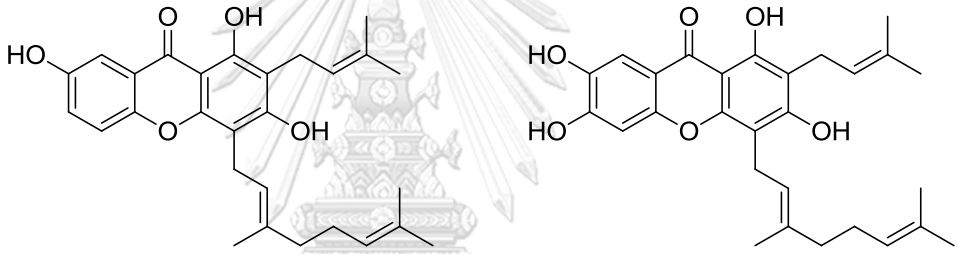
Species : *Cratoxylum cochinchinense*

Common name :Tui Kliang, Kheetui

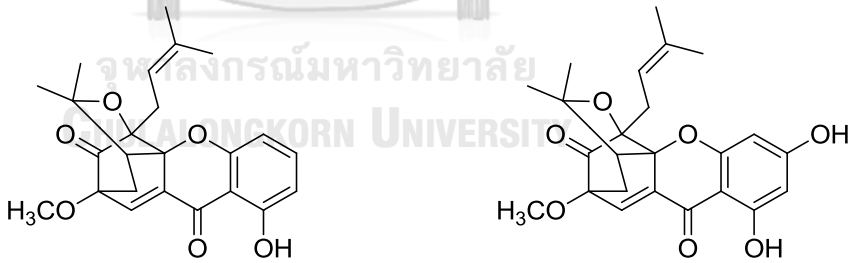
Local name : Tui Kliang

1.3 Chemical constituents and phytochemical investigation of *Cratoxylum cochinchinense*

In 2006, Mahabusarakum *et al*. [15] investigated the chemical constituents of the roots of *Cratoxylum cochinchinense.* Four new xanthones, including cochinchinone A, B, C, and D were isolated, along with eleven known compounds. The structures were elucidated on the basis of spectroscopic data interpretation. In addition, the isolated compounds were evaluated for DPPH radical scavenging activity. Cochinchinone B exhibited the most potent radical scavenging activity with IC_{50} values of 9.4 μM.



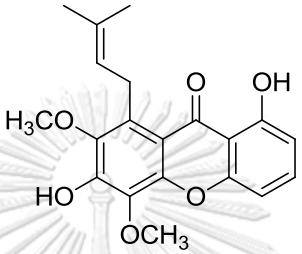
cochinchinone A cochinchinone B



cochinchinone C cochinchinone D

Figure 1.4 Chemical constituents from the roots of *Cratoxylum cochinchinense*

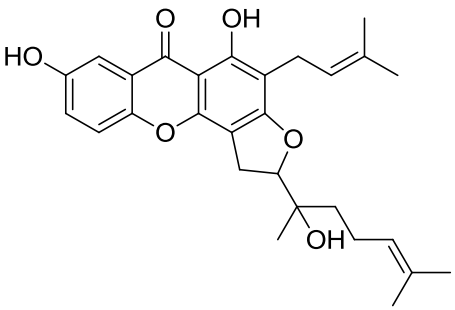
Laphookhieo *et al*. [16] in 2006 succeeded in isolating seven compounds, including one new as 5-*O*-methylcelebixanthone, along with six known compounds, celebixanthone, 1,3,7-trihydroxy-2,4-di(3-methylbut-2-enyl) xanthone, cochinchinone A, ***α***-mangostin, ***β***-mangostin, and cochinchinone C. The structures were elucidated on the basis of spectroscopic analysis. Moreover, all isolated compounds were evaluated for their cytotoxicity against NCI-H187 cells and antimalarial.



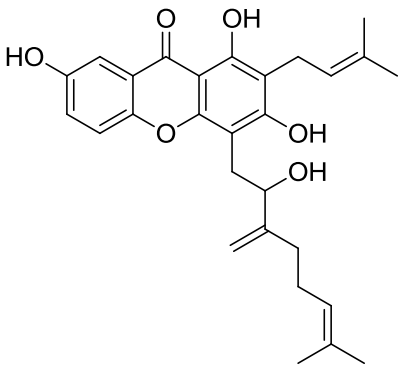
5-*O*-methylcelebixanthone

Figure 1.5 Chemical constituents from the roots of *Cratoxylum cochinchinense*

In 2018, Peng *et al*. [17] successfully isolated two new xanthones; cratoxanthone E and cratoxanthone F, along with ten known compounds from the methanol extract of the bark of roots of *Cratoxylum cochinchinense*. Their structures were characterized by spectroscopic methods. In addition, all isolated compounds were evaluated for their Protein tyrosine phosphatase 1B (PTP1B) and ***α***-glucosidase, two new xanthones showed a potent for both bioactivities.



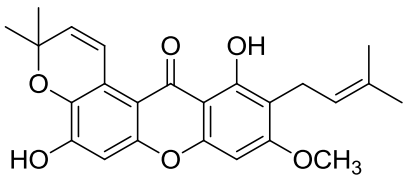
cratoxanthone E



cratoxanthone F

Figure 1.6 Chemical constituents from the bark of roots of *Cratoxylum cochinchinense*

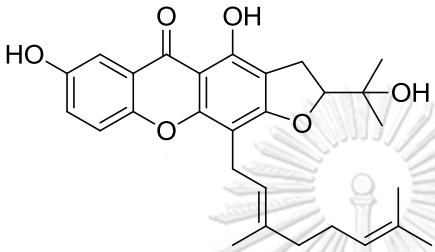
In 2006 Phuwaprasirisan *et al*. [13] isolated a new xanthone, cratoxylumxanthone A, together with five known xanthones, including dulcisxanthone B, **α**-mangostin, 2-geranyl-1,3,7-trihydroxy-4-(3-methylbut-2-enyl), and tectochrystin from the stem of *Cratoxylum cochinchinense*. The structure of isolated compounds were elucidated on the basis of spectroscopic analysis and their antioxidative on DPPH radical scavenging activity and lipid peroxidation. dulcisxanthone showed significant DPPH radical scavenging activity and lipid peroxidation with IC_{50} values of 0.39 and 0.024 mM.



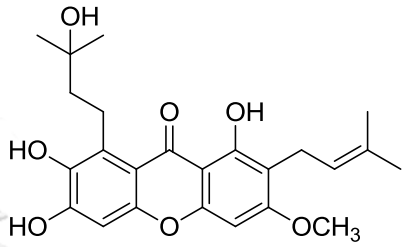
cratoxylumxanthone A

Figure 1.7 Chemical constituents from the stem of *Cratoxylum cochinchinense*

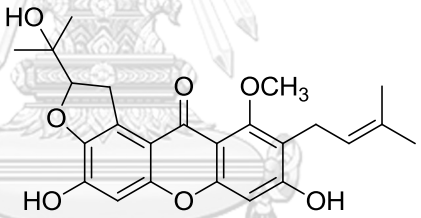
In 2012 Udomchotphruet *et al*. [18] studied the chemical constituents of the stem of *Cratoxylum cochinchinense*. Three new xanthones, cratoxylumxanthone B, cratoxylumxanthone C, and cratoxylumxanthone D, were isolated together with 5 known compounds. All structures of the isolated compounds were determined by spectroscopic methods. In addition, cratoxylumxanthone C showed significant DPPH radical scavenging activity and lipid peroxidation.



cratoxylumxanthones B



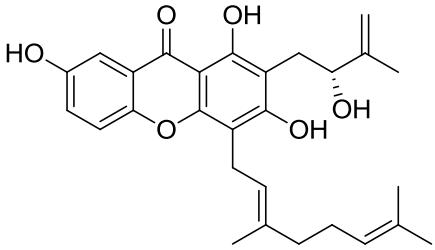
cratoxylumxanthones C



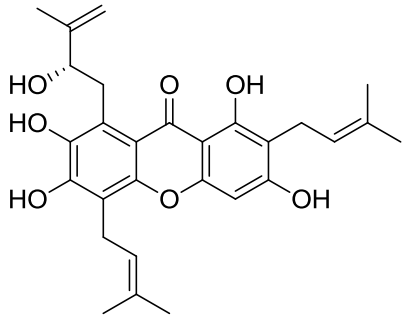
cratoxylumxanthones D

Figure 1.8 Chemical constituents from the stem of *Cratoxylum cochinchinense*

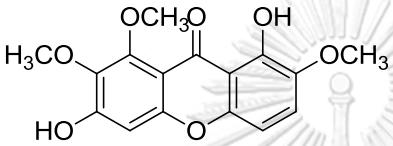
In 2017 Ito *et al*. [19] reported the isolated of four new xanthones, including cratoxanthone A, B, C, and D, along with six known compounds from the twigs and branches of *Cratoxylum cochinchinense*. The structure elucidation of the isolated compounds were achieved with the aid of extensive 1D and 2D NMR studies. The isolated compounds were evaluated for their cytotoxicity against NALM-6 cell lines, cratoxanthone A and B showed a potent cytotoxicity against NALM-6 cell lines with IC_{50} values of 1.98 μM.



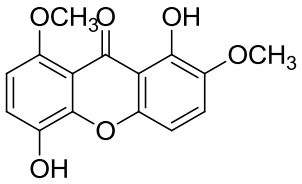
cratoxanthone A



cratoxanthone B



cratoxanthone C



cratoxanthone D

Figure 1.9 Chemical constituents from the twigs and branches of *Cratoxylum cochinchinense*

1.4 Cytotoxic activity against human cancer cell lines

Cancer is one of dangerous diseases caused by uncontrolled growth of cells. The proliferation of cancer cells may invade the other tissues and organs, and disrupt the metabolic pathways of normal cells. The discovery of anticancer agent from natural products has been developed initially though a preliminary screening of drug candidates. The MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide) assay is one of initial methods to screen the cytotoxicity of substance indicated by viability of the cells. The number of viable cells are determined through the reduction of MTT reagent by mitochondrial dehydrogenase enzyme inside living cells forming a formazan dye (**Figure 1.10**) which is measured then using colorimetric method. The result of cytotoxic activity can be used for further investigation through *in vivo* test using an animal model to assess the metabolism properties of a drug candidate in a living organism [20].

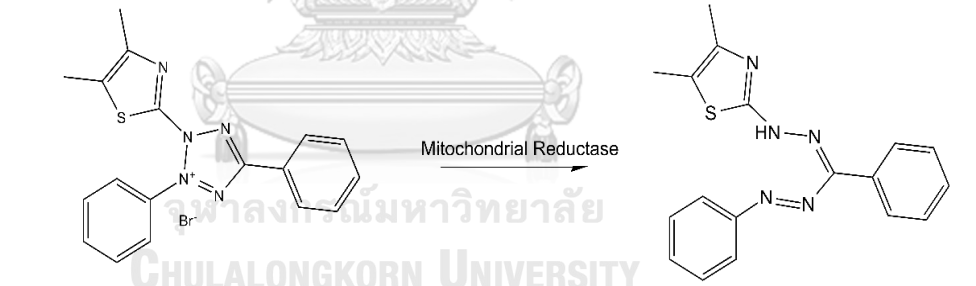


Figure 1.10 MTT reduction in live cells by mitochondrial reductase resulting a formation of insoluble formazan

From the literature review above showed a few reports on chemical constituent and their biological activities from the roots of *C. cochinchinense*. Therefore, those provide an insight to further investigation the bioactive compounds from the roots of this plant.

1.5 The objectives of this research

The main objectives in this investigation are as follows:

1. To isolate and purify, the compound from the roots of *C. cochinchinense*
2. To elucidate structurally the isolated compounds by means of spectroscopic analysis, including UV, IR, 1D and 2D NMR, and HRMS.
3. To evaluate the cytotoxicity of the isolated compounds against human cancer cell lines.

CHAPTER II

EXPERIMENTAL

Figure 2.1 The roots of *Cratoxylum cochinchinense*

2.1 Plant material

The roots of *C. cochinchinense* were collected in Lumpang Province, northern Thailand, in April 2018 and identified by Dr. Suttira Sedlak, a botanist at the Walai Rukhavej Botanical Research Institute, Mahasarakham University and a specimen retained as a reference (Khumkratok no. 01–18).

2.2 General experiment procedures

NMR spectra were recorded on Bruker 400 AVANCE spectrometer and JEOL RESONANCE 500 spectrometer. HRESIMS spectra were obtained using a Bruker MICROTOF model mass spectrometer. The UV–Visible absorption spectra were recorded on a UV-2550 UV–Vis spectrometer (Shimadzu, Kyoto, Japan). The IR spectra were measured on a Nicolet 6700 FT-IR spectrometer using KBr discs. Silica gel 60 Merck cat. Nos. 7734 and 7749 was used as absorbent for open column chromatography (CC) and radial chromatography (Chromatotron®), respectively. Thin layer chromatography (TLC) was performed on pre-coated Merck silica gel 60 F254 plates (0.25 mm thick layer) and visualized with 10% H_2SO_4–MeOH solution.

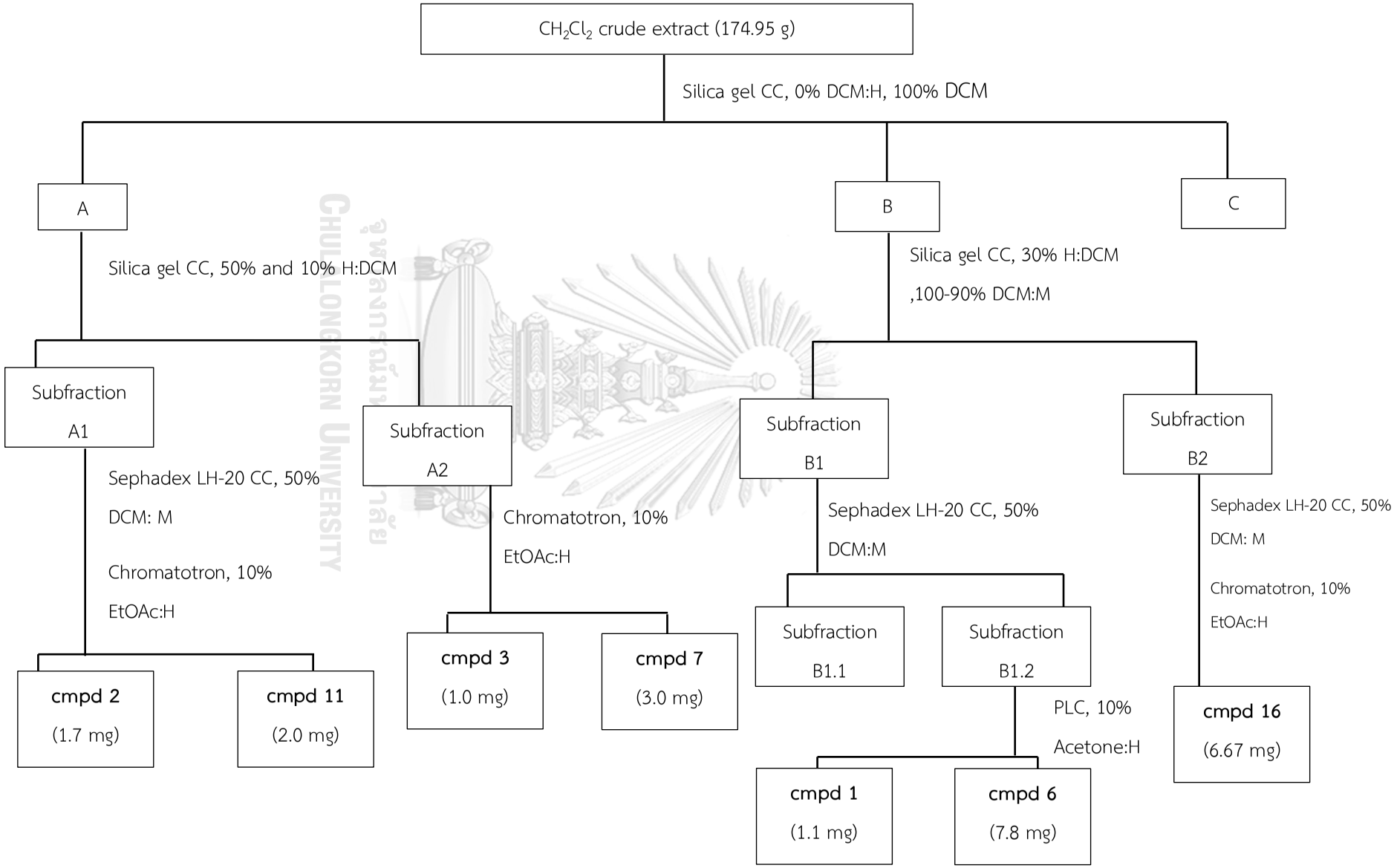
2.3 Extraction and isolation

The roots of *C. cochinchinense* (7.6 kg) were extracted with CH_2Cl_2 (3 × 5 L) at room temperature for 1 week. The combined extract was evaporated to give a yellowish-brown gum (174.95 g) was subjected to silica gel CC using a system of hexane (2 L), gradient of hexane–CH_2Cl_2 90, 80, 60, and 20% (5 L) and gradient of CH_2Cl_2–MeOH 95, 90, 80, 60, and 40% (5 L) yielding eight fractions (A–G). Fraction A (13 g) was subjected to silica gel CC using a system of 50 and 10% hexane–CH_2Cl_2 (800 mL) to provide two subfractions (A1-A2). Subfraction A1 was applied to Sephadex LH-20 CC, 50% of CH_2Cl_2-MeOH and further purified by chromatotron with a system of 10% EtOAc-hexane (500 mL each) to yield **2** (1.7 mg) and **11** (2.0 mg). Subfraction A2 was subjected to chromatotron with a system of 10% EtOAc-hexane to give compounds **3** (1.0 mg).and **7** (3.0 mg). Fraction B (8.0 g) was purified over silica gel CC using a system of 30% hexane-CH_2Cl_2 and gradient of CH_2Cl_2-MeOH 100, 95, and 90% (1 L) to give two subfractions (B1-B2). Then subfraction B1 (3 g) was also applied to a Sephadex LH-20 CC eluted with a system of 50% CH_2Cl_2-MeOH (900 mL each) followed by preparative thin layer chromatography (PTLC) with a system of 10% hexane–EtOAc (500 mL each) to give **1** (1.1 mg) and **6** (7.8 mg). Compound **16** (6.67 mg) was obtained from subfraction B2 (1.5 g) by Sephadex LH-20 CC eluted

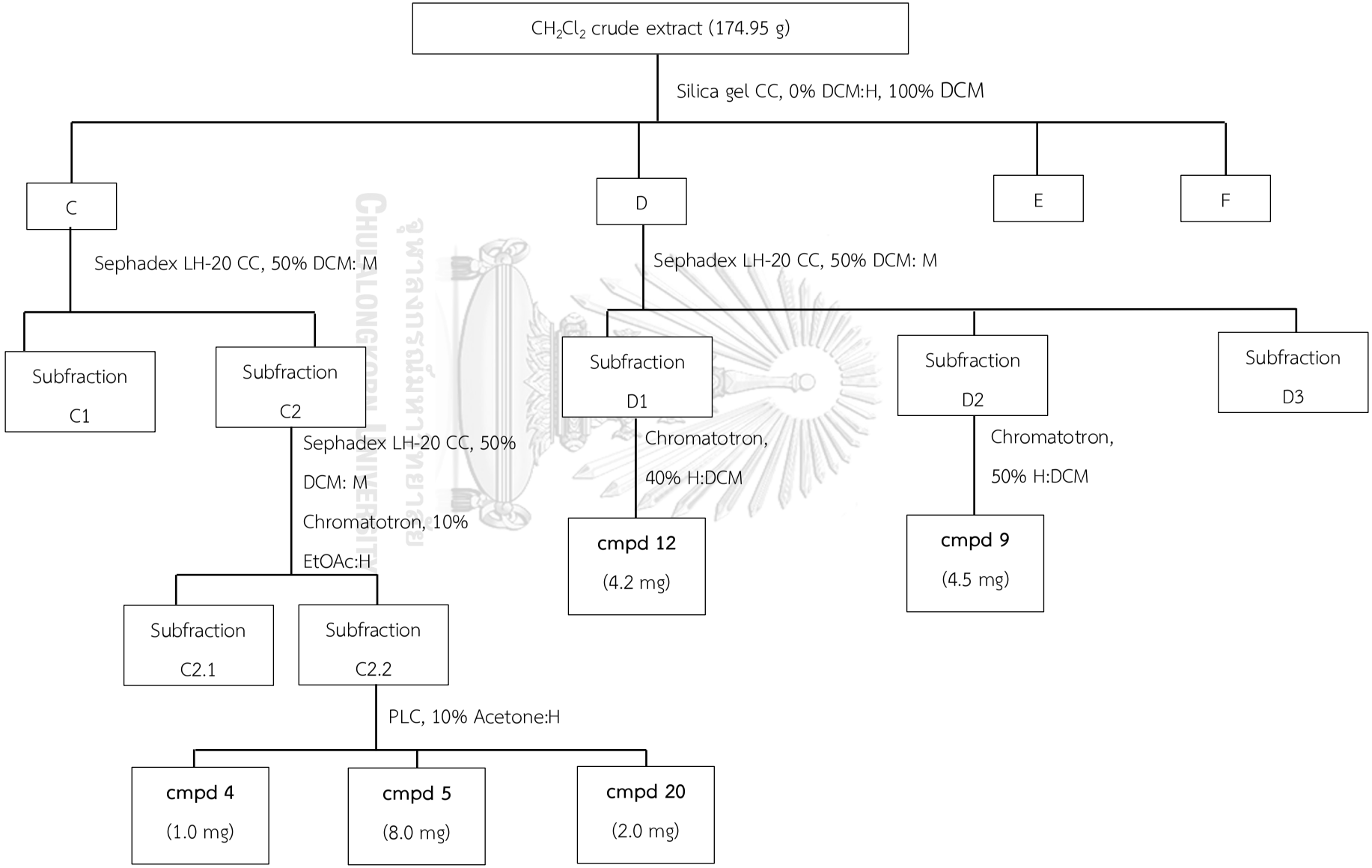
with a system of 50% CH_2Cl_2-MeOH (500 mL each) and chromatotron with a system of 10% EtOAc-hexane (100 mL). Fraction C (4.4 g) was separated by Sephadex LH-20 CC with a system of 50% CH_2Cl_2-MeOH (500 mL) and further applied to a chromatotron with a system of 20% EtOAc-hexane (200 mL) to provide subfractions (C1-C2). Subfraction C2 (2.0 g) was purified with PTLC using a system of 10% acetone-hexane (100 mL each) to furnish **4** (1.0 mg), **5** (8.0 mg), and **20** (2.0 mg). Fraction D (8.5 g) was purified by Sephadex LH-20 column eluted with 50% CH_2Cl_2-MeOH (2 L) to give three subfractions (D1-D3). Then Subfraction D1 (3.2 g) was purified by chromatotron with a system of 40% hexane-CH_2Cl_2 (200 mL) to obtain compounds **12** (4.2 mg) and subfraction D2 (2.2 g) was applied to chromatotron with a system of 50% hexane-CH_2Cl_2 (200 mL) to yield **9** (4.5 mg). Fraction E (3.0 g) was subjected to Sephadex LH-20 CC eluted with a system of 50% CH_2Cl_2-MeOH (400 mL each) and it was also purified by chromatotron with a system of 80% hexane-CH_2Cl_2 (300 mL) to afford three subfractions (E1-E3). Subfraction E2 was applied to chromatotron with a system of 80% hexane-CH_2Cl_2 (100 mL) to give compounds **8** (3.0 mg) and **13** (2.5 mg). Compound **18** (3.2 mg) was obtained from subfraction E3 by chromatotron with a system of 50% hexane-CH_2Cl_2 (100 mL). Fraction F (5.8 g) was separated by silica gel CC using isocratic elution of 50% hexane–CH_2Cl_2
(1 L) and using a system of 100, 95, and 90%, CH_2Cl_2–MeOH (1 L) to give compounds **10** (3.64 mg) and **14** (2.5 mg). Then it was also applied to chromatotron with a system of 80% hexane–CH_2Cl_2 (300 mL) to obtain compound **19** (4.3 mg). Finally, fraction G (2.2 g) was subjected to silica gel CC elution with 30% EtOAc-hexane (1L) and further applied to a chromatotron with 10% EtOAc-hexane (200 mL) to yield compounds **9** (3.7 mg), **15** (3.6 mg), and **17** (4.9 mg), respectively.

The isolated compounds were identified by means of various spectroscopic methods including MS, 1D and 2D NMR techniques as well as comparison with the previous literature for known compounds.

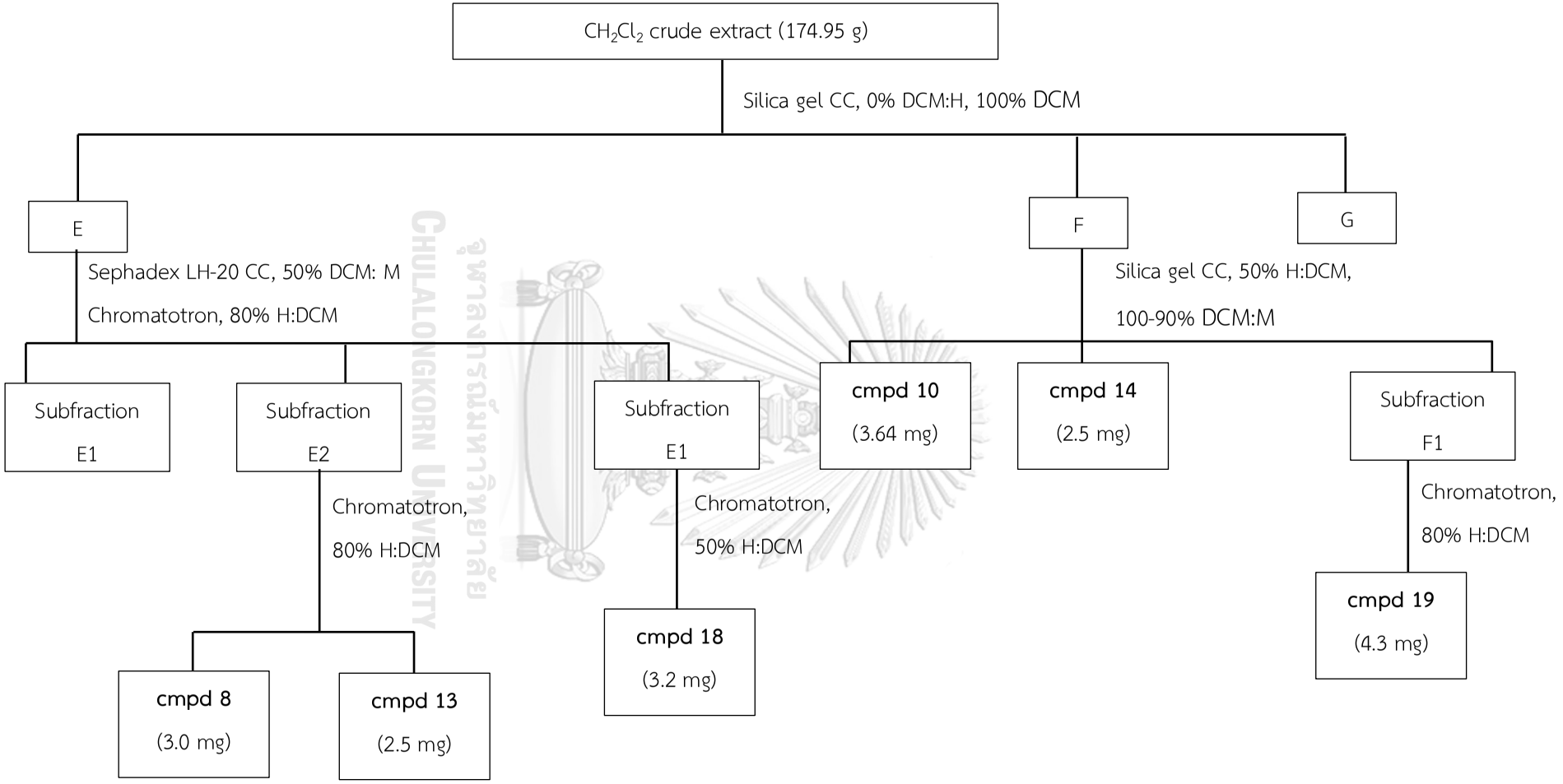
The isolation and purification of all isolated compounds from the roots of *C. cochinchinense* were briefly summarized in **Schemes 2.1**, **2.2**, **2.3**, and **2.4**.



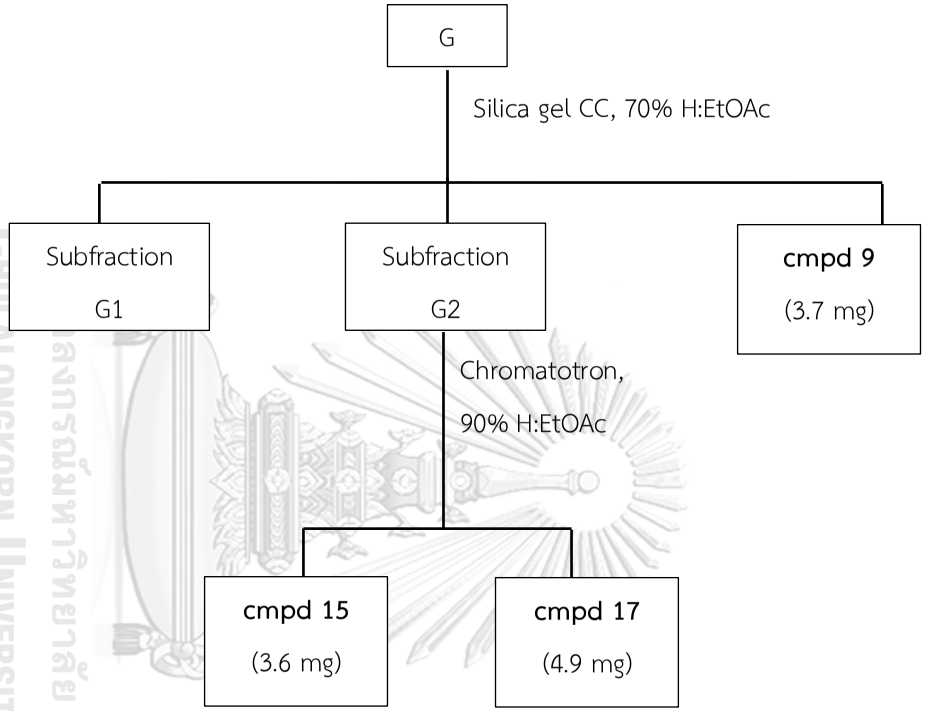
Scheme 2.1 Isolation procedure of subfractions A and B from the CH_2Cl_2 crude extract of *C. cochinchinense* roots



Scheme 2.2 Isolation procedure of subfractions C and D from the CH_2Cl_2 crude extract of *C. cochinchinense* roots



Scheme 2.3 Isolation procedure of subfractions E and F from the CH_2Cl_2 crude extract of *C. cochinchinense* roots



Scheme 2.4 Isolation procedure of subfraction G from the CH_2Cl_2 crude extract of *C. cochinchinense* roots

2.4 Cytotoxic activity against human cancer cell lines procedure

All isolated compounds (**1**-**22**) were applied to cytotoxic evaluation against KB, HeLa S-3, HT29, HepG2 cell lines employing the colorimetric method [21]. Doxorubicin was used as the reference substance which exhibits activity against five human cancer cell lines. The 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (Sigma Chemical Co., USA) was dissolved in saline to make a 5 mg/mL stock solution. Cancer cells (3x103 cells) suspended in 100 µg/wells of MEM medium containing 10% fetal calf serum FCS, Gibco BRL, Life Technologies, NY, USA) were seeded onto a 96-well culture plate (Coster, Corning Incorporated, NY 14831, USA). After 72 h pre-incubation at 37ºC in a humidified atmosphere of 5% CO_2/95% air to allow cellular attachment, various concentrations of test solution (10 µL/well) were added and these were then incubated for 48 h under the above conditions. At the end of the incubation at 37ºC for 4 h. The supernatant was decanted, and DMSO (100 µL/well) was added to allow formosan solubilization. The optical density (OD) of each well was detected using Microplate reader at 550 nm and for correction at 595 nm. Each determination represented the average mean of six replicates. The 50% inhibition concentration (IC_{50} value) was determined by curve fitting.

CHAPTER III
RESULTS AND DISCUSSION

3.1 Properties and structural elucidation of isolated compounds

The roots *C. cochinchinense* were grounded and extracted with CH_2Cl_2 at room temperature for 2 weeks. The CH_2Cl_2 crude extract was further subjected by various chromatographic methods using silica gel and Sephadex LH-20 as stationary phases to afford four new xanthones, cratochinone A-D (**1**-**4**) along with sixteen known xanthones (**5**-**20**), including pancixanthone-A (**5**), neriifolone A (**6**), macluraxanthone (**7**), 10-*O*-methyxlmacluraxanthone (**8**), pruniflorone G (**9**), pruniflorone H (**10**), 6-deoxyjacareubin (**11**), 9-hydroxycalabaxanthone (**12**), cratoxylumxanthone A (**13**), formoxanthone B (**14**) cochinchinone J (**15**), cochinchinone A (**16**), β-mangostin (**17**), 3,8-dihydroxy-1,2-dimethoxyxanthone (**18**), 1,5-dihydroxy-6-methoxyxanthone (**19**) and and 1,3,7-trihydroxyxanthone (**20**). The structures of all isolated compounds were characterized using spectroscopic method especially, NMR spectroscopies, as well as comparison with the previously reported in the literature.

3.1.1 Cratochinone A (1)

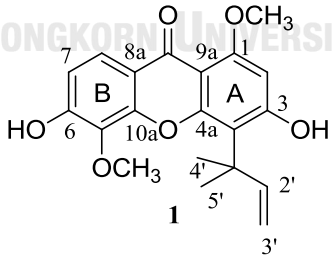


Figure 3.1 The chemical structure of compound 1

Cratochinone A (**1**) was obtained as a yellow gum. Its molecular formula was determined as $C_{20}H_{20}O_6$ by HRESIMS data (m/z 379.1148 $[M + Na]^+$, calcd. for $C_{20}H_{20}O_6Na$, 379.1158). The UV spectrum displayed absorption bands at λ_{max} 394, 315, and 244 nm. The IR spectrum showed phenolic hydroxyl groups and carbonyl group at 3432 and 1642 cm^{-1}. The ^1H NMR spectrum displayed a signal for aromatic

proton at δ_H 6.40 (1H, *s*, H-2) and two ortho-coupled aromatic protons at δ_H 6.99 (1H, *d*, *J* = 8.8, H-7) and δ_H 7.94 (1H, *d*, *J* = 8.8, H-8). In the HMBC spectrum (**Table 3.1, Figure. 3.1**), three aromatic protons were located at C-2, C-7, and C-8 by the correlation of δ_H 6.40 to δ_C 166.1 (C-1), δ_C 162.8 (C-3), δ_C 114.2 (C-4), and δ_C 103.8 (C-9a), δ_H 6.99 to δ_C 134.5 (C-5), δ_C 155.4 (C-6) and δ_C 115.1 (C-8a), and δ_H 7.94 to C-6, δ_C 181.29 (C-9), and δ_C 150.3 (C-10a), respectively. In addition, the splitting pattern and coupling constants of three olefinic protons at δ_H 6.30 (*dd*, *J* = 17.2, 10.7 Hz, H-2′), 4.85 (*d*, *J* = 17.2 Hz, H-3′a), and 4.85 (*d*, *J* = 10.7 Hz, H-3′b) indicated the presence of a terminal alkene as a part of a 1,1-dimethylallyl group which also displayed two singlets for methyl groups at δ_H 1.70 (each 3H, *s,* H-4′ and H-5′). The correlations of δ_H 6.30 to C-4, two methyl protons at δ_H 1.70 to δ_C 114.2 (C-4) and δ_C 151.0 (C-2′), confirming that a 1,1-dimethylallyl group was connected at C-4 of ring A. The ^{1}H and ^{13}C NMR spectroscopic data (**Table 3.1**) were shown to be similar to those of the known xanthone, isocudraniaxanthone A [22], except that the hydroxyl groups at C-1 and C-5 of isocudraniaxanthone A were substituted by methoxy groups. On the basis of HMBC cross peak of **1** (**Figure. 3.2**), the methoxy protons at δ_H 3.96 (3H, *s*, 1-OCH_3) and 3.84 (3H, *s*, 5-OCH_3) showed a cross peak with C-1 of ring A and C-5 of ring B, respectively. Thus, the completed assignment of cratochinone A was determined as **1**.

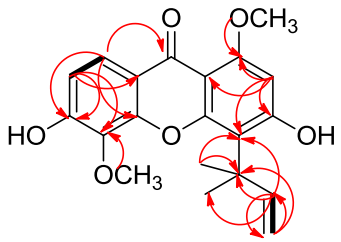


Figure 3.2 Selected HMBC (single headed arrow curves) and COSY (bold lines) correlations of **1**

Table 3.1 ^{1}H, ^{13}C and HMBC NMR data of **1** $CDCl_3$ (400 MHz for ^{1}H, 100MHz for ^{13}C)

Position	cratochinone A (**1**)		
	δ_H (*J* in Hz)	δ_c	HMBC correlations
1	-	166.1	-
2	6.40, (*s*)	96.4	C-1, C-3, C-4, C-9a
3	-	162.8	-
4	-	114.2	-
5	-	134.5	-
6	-	155.4	-
7	6.99, (*d*, 8.8)	113.5	C-5, C-6, C-8a
8	7.94, (*d*, 8.8)	122.7	C-6, C-9, C-10a
9	-	181.2	-
4a	-	157.6	-
8a	-	115.1	-
9a	-	103.8	-
10a	-	150.3	-
1’	-	41.7	-′
2’	6.30, (*dd*, 17.2, 10.7)	151.0	C-4, C-3′, C-4′, C-5′
3’	4.85, (*d*, 17.2)	108.0	C-1′, C-2′
	4.85, (*d*, 10.7)	108.0	
4’	1.70, (*s*)	30.4	C-4, C-1′, C-2′, C-5′
5’	1.70, (*s*)	30.2	C-4, C-1′, C-2′, C-4′
1-OCH_3	3.96, (*S*)	56.0	C-1
3-OH	-	-	-
5-OCH_3	3.84, (*S*)	62.8	C-5
6-OH	-	-	-

3.1.2 Cratochinone B (2)

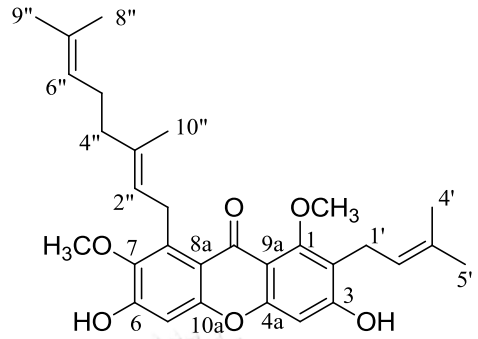


Figure 3.3 The chemical structure of compound **2**

Cratochinone B (**2**) was obtained as a brown gum. A molecular formula of $C_{30}H_{36}O_6$ was suggested by HRESIMS data (m/z = 493.2582 $[M + H]^+$, calcd for $C_{30}H_{37}O_6$, 493.2590). The UV spectrum displayed absorption bands at λ_{max} at 369, 315, 269, and 245 nm. The IR data indicated the presence of a xanthone skeleton at 3431, 1639, and 1610 cm^{-1}. The ^1H NMR spectrum displayed two aromatic protons at δ_H 6.35 (1H, *s*, H-4) and δ_H 6.84 (1H, *s*, H-5). The presence of a prenyl group was indicated by signals for an olefinic proton at δ_H 5.23 (1H, m, H-2′), methylene protons at δ_H 3.35 (2H, *d*, *J* = 7.2 Hz, H-1′), and two methyl groups at δ_H 1.68 (3H, *s*, H-4′) and 1.79 (3H, *s*, H-5′). The correlation of δ_H 3.35 to δ_C 163.9 (C-1), δ_C 111.9 (C-2) and δ_C 159.3 (C-3) in the HMBC spectrum (**Table 3.2, Figure. 3.3**) established that the prenyl unit was connected at C-2 of ring A.

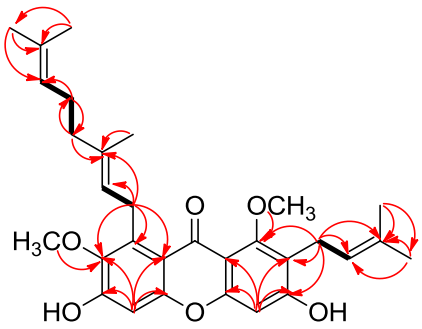


Figure 3.4 Selected HMBC (single headed arrow curves) and COSY (bold lines) correlations of **2**

Furthermore, a geranyl unit was identified from the resonances of two olefinic protons at δ_H 5.26 (1H, *m*, H-2″) and 5.10 (1H, *m*, H-6″), three methylene protons at δ_H 4.10 (2H, *d*, *J* = 7.2 Hz, H-1″), δ_H 2.03 (4H, *m*, H-4″ and H-5″), and three methyl groups at δ_H 1.83 (3H, *s*, H-10″), 1.68 (3H, *s*, H-8″), and 1.67 (3H, *s*, H-9″), which were also corroborated by the observed HMBC data (**Figure. 3.4**). The ^{1}H and ^{13}C NMR spectroscopic data (**Table 3.2**) were shown to be similar to those of the known xanthone, norcowanin [23], except that the hydroxyl group at C-1 was replaced by a methoxy group. In the HMBC correlations of **2** (**Figure. 3.4**), the methoxy proton at δ_H 3.90 (3H, *s*, 1-OCH_3) showed a cross peak with C-1. The remaining signals of methoxy proton at δ_H 3.80 (3H, *s*, 7-OCH_3), which were accommodated at δ_C 143.0 (C-7) of ring B were based on HMBC cross peak. From the above evidence, cratochinone B had the structure **2**.

Table 3.2 ^{1}H, ^{13}C and HMBC NMR data of **2** $CDCl_3$ (400 MHz for ^{1}H, 100MHz for ^{13}C)

Position	cratochinone B (**2**)		
	δ_H (*J* in Hz)	δ_c	HMBC correlations
1	-	163.9	-
2	-	111.9	-
3	-	159.3	-
4	6.35, (*s*)	89.2	C-2, C-3, C-4a, C-9a
5	6.84, (*s*)	101.9	C-6, C-7, C-8a, C-10a
6	-	155.4	-
7	-	143.0	-
8	-	137.8	-
9	-	183.2	-
4a	-	155.7	-
8a	-	112.2	-
9a	-	103.3	-
10a	-	154.9	-
1’	3.35, (*d*, 7.2)	21.8	C-1, C-2, C-3, C-2′, C-3′
2’	5.23, (*m*)	122.7	C-4′, C-5′
3’	-	131.6	-
4’	1.68, (*s*)	25.4	C-2′, C-5′
5’	1.79, (*s*)	18.6	C-2′, C-4′
1”	4.10, (*d*, 7.2)	26.2	C-7, C-8, C-8a, C-2″, C-3″
2”	5.26, (*m*)	125.4	C-9″
3”	-	135.7	-
4”	2.03, (*m*)	32.3	C-3″, C-5″
5”	2.03, (*m*)	27.2	C-3″, C-5″
6”	5.10, (*m*)	123.6	C-5″, C-8″, C-10″
7”	-	132.1	-
8”	1.83, (*s*)	23.4	C-6″, C-7″, C-10″
9”	1.68, (*s*)	14.5	C-2″, C-3″, C-4″
10”	1.67, (*s*)	18.6	C-6″, C-7″, C-8″
1-OCH_3	3.90, (*s*)	56.3	C-1
3-OH	-	-	-
6-OH	-	-	-
7-OCH_3	3.80, (*s*)	61.9	C-7

3.1.3 Cratochinone C (3)

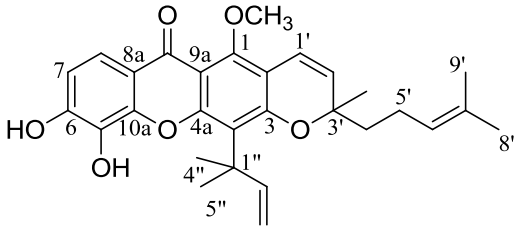


Figure 3.5 The chemical structure of compound **3**

Cratochinone C (**3**) was obtained as a brown gum. Its molecular formula was deduced as $C_{29}H_{32}O_6$ by HRESIMS data (m/z = 477.2270 $[M + H]^+$, calcd for $C_{29}H_{33}O_6$, 477.2232). The UV and IR spectrum displayed characteristic of xanthone skeleton. The ^{1}H NMR spectrum (**Table 3.3**) displayed signals of two ortho-coupled aromatic protons at δ_H 7.73 (1H, *d*, *J* = 8.8 Hz, H-7) and 6.95 (1H, *d*, *J* = 8.8 Hz, H-7). The occurrence of a chromene ring bearing a methyl group was inferred from signals at δ_H 6.75 (1H, *d*, *J* = 10.0 Hz, H-1'), 5.60 (1H, *d*, *J* = 10.0 Hz, H-2'), and two methyl group at δ_H 1.64 (3H, *s*, H-10'). and the six-carbon was displayed signals for an olefinic proton at δ_H 5.11 (1H, *m*, H-6'), methylene protons at δ_H 2.02 (each 2H, *d*, *J* = 7.2 Hz, H-4' and H5'), and two methyl groups at δ_H 1.64 (3H, *s*, H-9') and 1.49 (3H, *s*, H-8'). The location of a chromene ring was confirmed by HMBC (**Table 3.3**, **Figure. 3.6**), in which the methine proton H-1' at δ_H 6.75 was correlated with δ_C 157.2 (C-1), 159.3 (C-3) and 81.3 (C-3'), while the methine proton H-2' at δ_H 5.60 was correlated with δ_C 105.6 (C-2) and 41.5 (C-4').

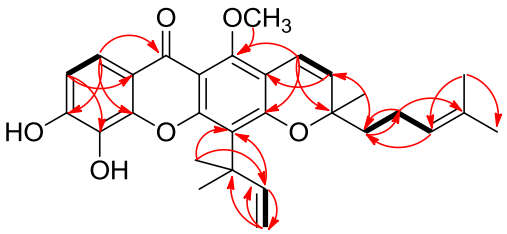


Figure 3.6 Selected HMBC (single headed arrow curves) and COSY (bold lines) correlations of **3**

In addition, the splitting pattern and coupling constants of three olefinic protons at δ_H 6.64 (1H, *d*, *J* = 17.4, 11.0 Hz, H-2”), 5.17 (1H, *d*, *J* = 17.4 Hz, H-3”a) and 5.03 (1H, *d*, *J* = 11.0 Hz, H-3”b) indicated the presence of terminal alkene as a part of 1,1-dimethylallyl group which also displayed signals of two methyl group at δ_H 1.64 (each 3H, *s*, H-4” and H-5”). The correlation of δ_H 6.64 to δ_C 114.4 (C-4) and 104.8 (C-3”) in the HMBC spectrum (**Table 3.3, Figure. 3.6**) established that the 1,1-dimethylallyl group was connected at C-4 of ring A. The ^{1}H and ^{13}C NMR spectroscopic data (**Table 3.2**) were shown to be similar to those of the known xanthone, pruniflorone H [24], except that the hydroxyl group at C-1 was replaced by a methoxy group. In the HMBC correlations of **3** (**Figure. 3.6**), the methoxy proton at δ_H 3.90 (3H, *s*, 1-OCH_3) showed a cross peak with C-1. From this data, the structure of **3** suggested that compound **3** was cratochinone C.

Table 3.3 ^{1}H, ^{13}C and HMBC NMR data of **3** $CDCl_3$ (400 MHz for ^{1}H, 100MHz for ^{13}C)

Position	cratochinone C (**3**)		
	δ_H (*J* in Hz)	δ_c	HMBC correlations
1	-	157.2	-
2	-	105.6	-
3	-	159.3	-
4	-	114.4	-
5	-	133.7	-
6	-	151.6	-
7	6.95, (*d*, 8.8)	108.6	C-5, C-8a
8	7.73, (*d*, 8.8)	116.9	C-6, C-9, C-10a
9	-	181.1	-
4a	-	154.6	-
8a	-	113.5	-
9a	-	108.7	
10a	-	144.5	-
1’	6.75, (*d*, 10)	116.3	C-1, C-3, C-3′
2’	5.60, (*d*, 10)	127.3	C-2′, C-1′, C-3′, C-4′
3’	-	81.3	-
4’	2.02, (*m*)	41.5	C-2′, C-5′
5’	2.02, (*m*)	24.2	C-3′, C-7′
6’	5.11, (*m*)	125.7	C-4′, C-5′, C-8′, C-9′
7’	-	130.5	-
8’	1.49, (*s*)	18.1	C-6′, C-7′, C-9′
9’	1.49, (*s*)	29.8	C-6′, C-7′, C-8′
10’	1.64, (*s*)	25.8	C-2′, C-4′
1”	-	41.6	-
2”	6.64 (*dd*, 17.6, 10.6)	155.1	C-4, C-1″, C-3″
3”	5.17, (*d*, 17.6)	104.9	C-2″, C-4″, C-5″
	5.03, (*d*, 17.6)		
4”	1.66, (*s*)	28.6	C-4″, C-5″
5”	1.66, (*s*)	28.0	C-4″, C-4″
1-OCH_3	4.01, (*s*)	62.44	C-1
5-OH	-	-	-
6-OH	-	-	-

3.1.4 Cratochinone D (4)

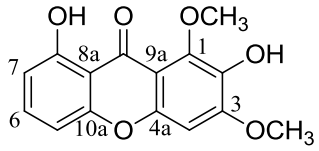


Figure 3.7 The chemical structure of compound **4**

Cratochinone D (**4**) was obtained as a yellow powder. Its molecular formula was determined as $C_{15}H_{12}O_6$ by HRESIMS measurement through the molecular ion peak at m/z 311.0542 $[M + Na]^+$ (calcd for $C_{15}H_{12}O_6Na$, 311.0542). The UV spectrum displayed absorption bands at λ_{max} 312, 265 and 245 nm, which is typical of xanthone chromophore. The IR spectrum showed O-H and C=O stretching bands at 3208 and 1678 cm^{-1}. The 1H NMR spectrum displayed signals for a chelated hydroxy proton at δ_H 13.12 (1H, *s*, H-8) and aromatic proton singlet at δ_H 6.78 (1H, *s*, H-4). Moreover, the splitting pattern and coupling constants of a 1,2,3-trisubstituted benzene moiety in ring B were observed at δ_H 7.50 (1H, *t*, *J* = 8.0 Hz, H-6), 6.83, (1H, *dd*, *J* = 8.0, 2.0 Hz, H-5) and 6.75, (1H, *dd*, *J* = 8.0, 2.0 Hz, H-7). The methoxy protons at δ_H 3.96 (3H, *s*, 1-OCH_3) and 3.84 (3H, *s*, 5-OCH_3) showed a cross peak with C-1 of ring A and C-5 of ring B, respectively (Figure 3.8). Thus, the structure of cratochinone D was assigned as **4**.

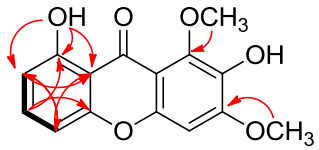


Figure 3.8 Selected HMBC (single headed arrow curves) and COSY (bold lines) correlations of **4**

Table 3.4 ^{1}H, ^{13}C and HMBC NMR data of **4** $CDCl_3$ (400 MHz for ^{1}H, 100MHz for ^{13}C)

Position	cratochinone D (**4**)		
	δ_H (*J* in Hz)	δ_c	HMBC correlations
1	-	152.3	-
2	-	137.5	C-1, C-3, C-4, C-9a
3	-	155.9	-
4	6.78, (*s*)	99.1	-
5	6.83, (dd, 8.0, 2.0)	106.3	-
6	7.50, (*t*, 8.0)	136.0	-
7	6.75, (dd, 8.0, 2.0)	108.8	C-5, C-6, C-8a
8	-	162.1	C-6, C-9, C-10a
9	-	181.5	-
4a	-	154.7	-
8a	-	110.7	-
9a	-	109.3	-
10a	-	155.6	-
1-OCH_3	4.01, (*s*)	61.8	C-1
2-OH	-	-	-
3-OCH_3	4.03, (*s*)	56.3	C-3
8-OH	13.12, (*s*)	-	C-7, C-8, C-8a

3.1.5 Pancixanthone-A (5)

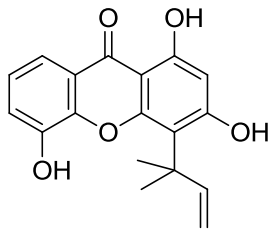


Figure 3.9 The chemical structure of compound **5**

Pancixanthone-A (**5**) (**Figure 3.9**) The structure of compound 5 was determined and confirmed by comparison of the physical and spectroscopic data with a previous report [25].

3.1.6 Neriifolone A (6)

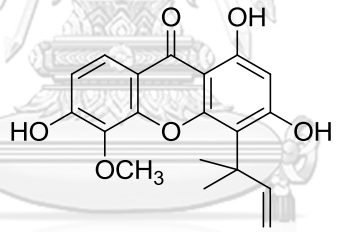


Figure 3.10 The chemical structure of compound **6**

Neriifolone A (**6**) (**Figure 3.9**) The structure of compound 6 was determined and confirmed by comparison of the physical and spectroscopic data with those of neriifolone A reported in the literature [26].

3.1.7 Macluraxanthone (7)

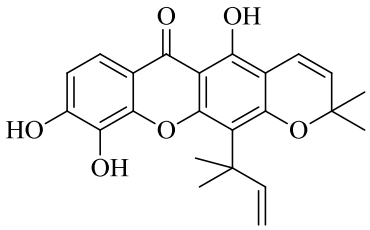


Figure 3.11 The chemical structure of compound **7**

Macluraxanthone (**7**) (**Figure 3.11**) The structure of compound **6** was determined and confirmed by comparison the physical and the ^{1}H and ^{13}C NMR data of compound **6** with those of macluraxanthone reported in the literature [27].

3.1.8 10-*O*-methyxlmacluraxanthone (8)

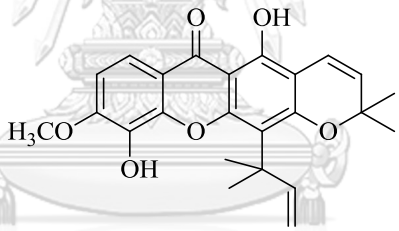


Figure 3.12 The chemical structure of compound **8**

10-*O*-methyxlmacluraxanthone (**8**) (**Figure 3.12**) The ^{1}H and ^{13}C NMR data of compound **8** was identical to 10-*O*-methyxlmacluraxanthone by comparison of the physical and spectroscopic data with previous reported in the literature [28].

3.1.9 Pruniflorone G (9)

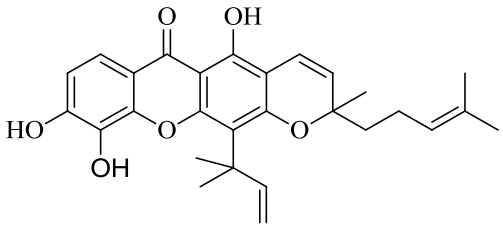


Figure 3.13 The chemical structure of compound **9**

Pruniflorone G (**9**) (**Figure 3.13**) The structure of compound **9** was determined and confirmed by comparison of the physical and spectroscopic data with a previous report [24].

3.1.10 Pruniflorone H (10)

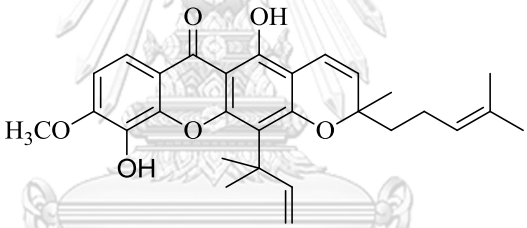


Figure 3.14 The chemical structure of compound **10**

Pruniflorone H (**10**) (**Figure 3.14**) The structure of compound **10** was determined and confirmed by comparison the physical and the ^{1}H and ^{13}C NMR data of compound **10** with those of pruniflorone H reported in the literature [24].

3.1.11 6-deoxyjacareubin (11)

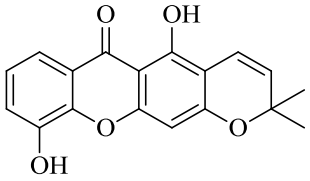


Figure 3.15 The chemical structure of compound **11**

6-deoxyjacareubin (**11**) (**Figure 3.14**) The ^{1}H and ^{13}C NMR data of compound **8** was identical to 6-deoxyjacareubin by comparison of the physical and spectroscopic data with previous reported in the literature [29].

3.1.12 9-hydroxycalabaxanthone (12)

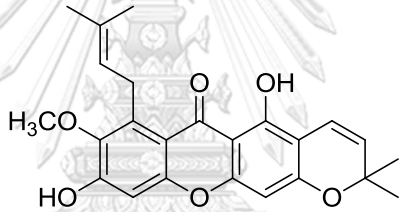


Figure 3. 16 The chemical structure of compound **12**

9-hydroxycalabaxanthone (**12**) (**Figure 3.16**) The structure of compound **12** was determined and confirmed by comparison the physical and the ^{1}H and ^{13}C NMR data of compound **12** with those of 9-hydroxycalabaxanthone reported in the literature [30].

3.1.13 Cratoxylumxanthone A (13)

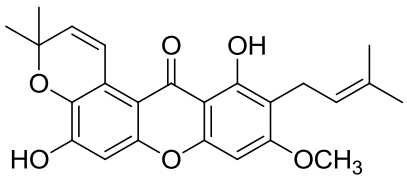


Figure 3.17 The chemical structure of compound **13**

Cratoxylumxanthone A (**13**) (**Figure 3.17**) The ^{1}H and ^{13}C NMR data of compound **13** was identical to cratoxylumxanthone A by comparison of the physical and spectroscopic data with previous reported in the literature [13].

3.1.14 Formoxanthone B (14)

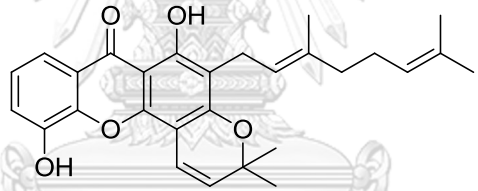


Figure 3. 18 The chemical structure of compound **14**

Formoxanthone B (**14**) (**Figure 3.18**) The structure of compound **14** was determined and confirmed by comparison the physical and the ^{1}H and ^{13}C NMR data of compound **14** with those of formoxanthone B reported in the literature [31].

3.1.15 Cochinchinone J (15)

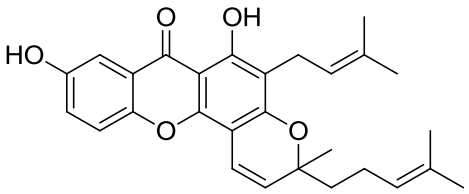


Figure 3.19 The chemical structure of compound **15**

Cochinchinone J (**15**) (**Figure 3.18**) The ^{1}H and ^{13}C NMR data of compound **15** was identical to cochinchinone J by comparison of the physical and spectroscopic data with previous reported in the literature [32].

3.1.16 Cochinchinone A (16)

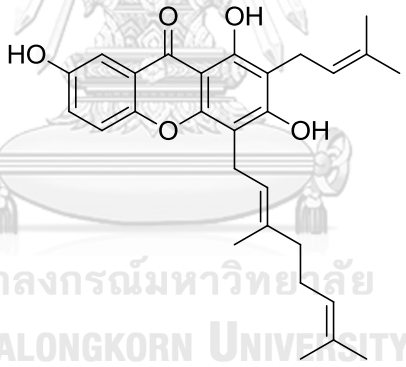


Figure 3.20 The chemical structure of compound **16**

Cochinchinone A (**16**) (**Figure 3.18**) The ^{1}H and ^{13}C NMR data of compound **16** was identical to cochinchinone A by comparison of the physical and spectroscopic data with previous reported in the literature [15].

3.1.17 *β*-mangostin (17)

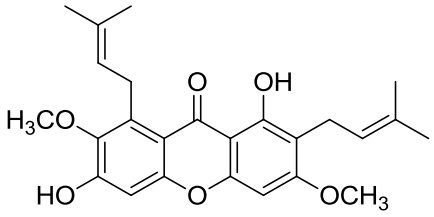


Figure 3.21 The chemical structure of compound **17**

β-mangostin (**17**) (**Figure 3.20**) The ^{1}H and ^{13}C NMR data of compound **17** was identical to *β*-mangostin by comparison of the physical and spectroscopic data with previous reported in the literature [33].

3.1.18 3,8-dihydroxy-1,2-dimethoxyxanthone (18)

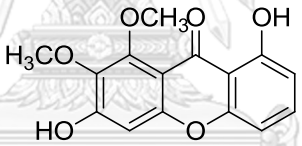


Figure 3.22 The chemical structure of compound **18**

3,8-dihydroxy-1,2-dimethoxyxanthone (**18**) (**Figure 3.21**) The ^{1}H and ^{13}C NMR data of compound **16** was identical to 3,8-dihydroxy-1,2-dimethoxyxanthone by comparison of the physical and spectroscopic data with previous reported in the literature [34].

3.1.19 1,5-dihydroxy-6-methoxyxanthone (19)

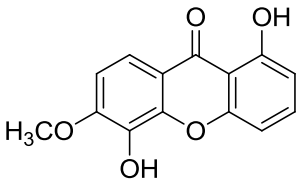


Figure 3.23 The chemical structure of compound **19**

1,5-dihydroxy-6-methoxyxanthone (**19**) (**Figure 3.22**) The ^{1}H and ^{13}C NMR data of compound **19** was identical to 1,5-dihydroxy-6-methoxyxanthone by comparison of the physical and spectroscopic data with previous reported in the literature [35].

3.1.20 1,3,7-trihydroxyxanthone (20)

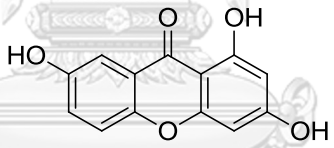


Figure 3.24 The chemical structure of compound **20**

1,3,7-trihydroxyxanthone (20) (**Figure 3.21**) The ^{1}H and ^{13}C NMR data of compound **20** was identical to 3,8-dihydroxy-1,2-dimethoxyxanthone by comparison of the physical and spectroscopic data with previous reported in the literature [36].

3.2 Cytotoxicity of isolated compounds (1-31) against human cancer cell lines

all isolated xanthones (1–18) were first evaluated *in vitro* for their cytotoxicity against KB and HeLa S-3 cells. The active compounds (**2**, **7**, **9**, **12**, and **14**) with IC_{50} values lower than 10 μM toward these two cancer cell lines were further evaluated against three cell lines, including HT-29, MCF-7, and Hep G2 cells. The results of cytotoxicity were shown in Table 3.5. Most of the tested compounds showed moderate to weak cytotoxicity, except **2**, **7**, and **9**, which showed significant cytotoxic activities against five human cancer cell lines with IC_{50} values in the range of 0.91–9.93 μM. Compound **12** exhibited potent cytotoxicity toward KB, HeLa S-3, and HT-29 cells with IC_{50} values of 7.39, 6.07, and 8.11 μM, respectively. Whereas **14** showed good cytotoxicity toward both KB and HeLa S-3 cells with IC_{50} values of 7.28 and 9.84 μM. Compounds **5**, **18**, **19**, and **20** showed inactive cytotoxicity toward both KB and HeLa S-3 cells with IC_{50} values more than 100 μM. In addition, two new xanthones, compound **3** and **4** were not tested on cytotoxic activities against five human cancer cell lines because limitation of the amount of substance. The SAR studied data (**Figure. 4.1; Table 3.5**) of xanthones suggest that the geranyl group at C-8 [37], the ortho hydroxy group at C-5 and C-6, and the 1,1-dimethylallyl group at C-4 [38], might improve the cytotoxicity as inferred from the comparison of their cytotoxicity of xanthones **1**–**20**.

Table 3.5 Cytotoxicity of isolated compounds (**1**-**20**) from *C. cochinchinense* roots

Compounds	IC_{50} (μM) ± SD				
	KB	Hela S-3	HT-29	MCF-7	Hep G2
1	42.17 ± 2.83	58.41 ± 0.51	N.T.	N.T.	N.T.
2	1.54 ± 0.02	0.91 ± 0.21	7.04 ± 0.83	1.76 ± 0.06	1.72 ± 0.10
3	N.T.	N.T.	N.T.	N.T.	N.T.
4	N.T.	N.T.	N.T.	N.T.	N.T.
5	> 100	> 100	N.T.	N.T.	N.T.
6	10.14 ± 0.10	12.62 ± 0.85	N.T.	N.T.	N.T.
7	1.60 ± 0.02	1.85 ± 0.19	8.58 ± 0.14	1.18 ± 0.04	9.57 ± 0.74
8	53.01 ± 4.88	35.12 ± 2.63	N.T.	N.T.	N.T.
9	2.04 ± 0.04	2.681 ± 0.10	9.93 ± 0.52	2.54 ± 0.22	4.43 ± 0.56
10	46.11 ± 1.17	40.32 ± 2.62	N.T.	N.T.	N.T.
11	28.01 ± 0.84	13.42 ± 0.91	N.T.	N.T.	N.T.
12	7.39 ± 0.15	6.07 ± 0.59	8.11 ± 0.43	13.67 ± 0.31	27.72 ± 0.61
13	26.44 ± 2.61	10.50 ± 0.86	N.T.	N.T.	N.T.
14	7.28 ± 0.56	9.84 ± 0.45	24.14 ± 0.7	19.63 ± 1.43	19.96 ± 0.94
15	42.18 ± 1.60	59.25 ± 0.14	N.T.	N.T.	N.T.
16	28.96 ± 0.10	20.54 ± 0.83	N.T.	N.T.	N.T.
17	24.99 ± 3.16	14.383 ± 2.67	N.T.	N.T.	N.T.
18	> 100	> 100	N.T.	N.T.	N.T.
19	> 100	> 100	N.T.	N.T.	N.T.
20	> 100	> 100	N.T.	N.T.	N.T.
Doxorubicin	0.22 ± 0.01	0.15 ± 0.05	0.59 ± 0.03	1.29 ± 0.02	0.99 ± 0.17

Note: $IC_{50} \leq 10$ μM = good activity, 10 μM < $IC_{50} \leq 30$ μM = moderate activity, 30 μM < $IC_{50} \leq 100$ μM = weak activity, $IC_{50} > 100$ μM = inactive.

N.T.; the compounds were not tested.

CHAPTER IV
CONCLUSION

In conclusion, compounds **1**-**20** were successfully isolated and purified from dichloromethane extracts of *C. cochinchinense* roots, which was fractionated through various chromatographic methods to afford four new xanthone derivatives, named cratochinone A (**1**) and cratochinone B (**2**), cratochinone C (**3**), cratochinone D (**4**), along with 16 known xanthones (**Figure. 4.1**), including pancixanthone-A (**5**), neriifolone A (**6**), macluraxanthone (**7**), 10-*O*-methyxlmacluraxanthone (**8**), pruniflorone G (**9**), pruniflorone H (**10**), 6-deoxyjacareubin (**11**), 9-hydroxycalabaxanthone (**12**), cratoxylumxanthone A (**13**), formoxanthone B (**14**) cochinchinone J (**15**), cochinchinone A (**16**), β-mangostin (**17**), 3,8-dihydroxy-1,2-dimethoxyxanthone (**18**), 1,5-dihydroxy-6-methoxyxanthone (**19**) and and 1,3,7-trihydroxyxanthone (**20**). The structures of all isolated compounds were characterized using spectroscopic method especially, 1D and 2D NMR as well as comparison of the ^{1}H and ^{13}C NMR with the previously reported in the literature. Moreover, the cytotoxic activity against KB and HeLa S-3 cancer cell lines were performed to evaluate the bioactivity of all isolated compounds.

The cytotoxicity of all isolated compounds (**1**-**20**) were first evaluated in vitro for their cytotoxicity against KB and HeLa S-3 cells. The active compounds (**2**, **7**, **9**, **12**, and **14**) with IC_{50} values lower than 10 μM toward these two cancer cell lines were further evaluated against three cell lines, including HT-29, MCF-7, and Hep G2 cells. Most of the tested compounds showed moderate to weak cytotoxicity, except **2**, **7**, and **9**, which showed significant cytotoxic activities against five human cancer cell lines with IC_{50} values in the range of 0.91–9.93 μM. Compound **12** exhibited potent cytotoxicity toward KB, HeLa S-3, and HT-29 cells with IC_{50} values of 7.39, 6.07, and 8.11 μM, respectively. Whereas **14** showed good cytotoxicity toward both KB and HeLa S-3 cells with IC_{50} values of 7.28 and 9.84 μM. Compounds **5**, **18**, **19**,

and **20** showed inactive cytotoxicity toward both KB and HeLa S-3 cells with IC_{50} values more than 100 μM. In addition, two new xanthones, compound **3** and **4** were not tested on cytotoxic activities against five human cancer cell lines because limitation of the amount of substance.

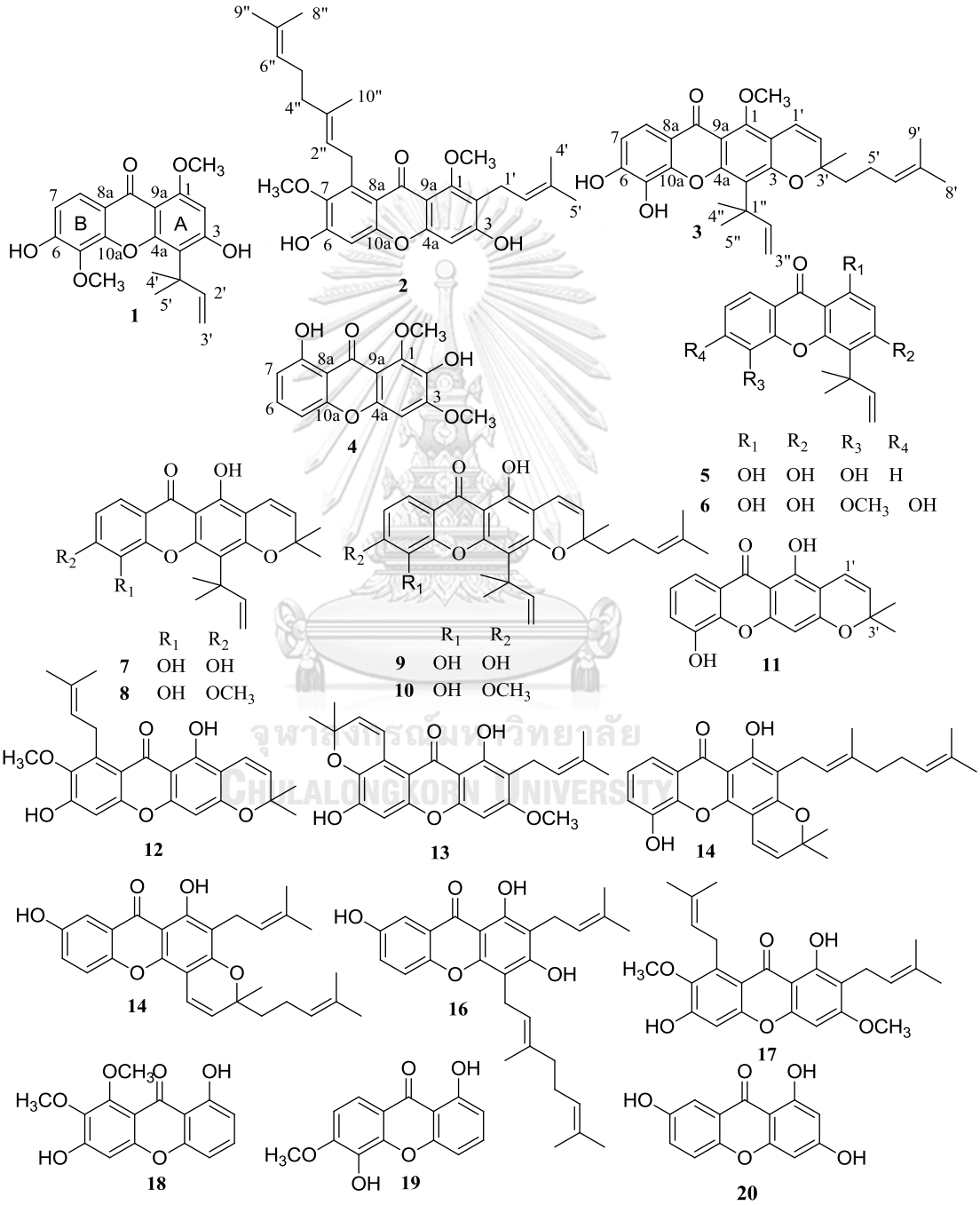


Figure 4.1 Structures of xanthones **1**-**20** from the roots of *C. cochinchinense*

The future works may involve the modification and synthesis of active compounds for a new potent drug. In addition, these results might provide basic knowledge to study the mechanism of active compounds toward disease for the drug improvement.

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APPENDIX

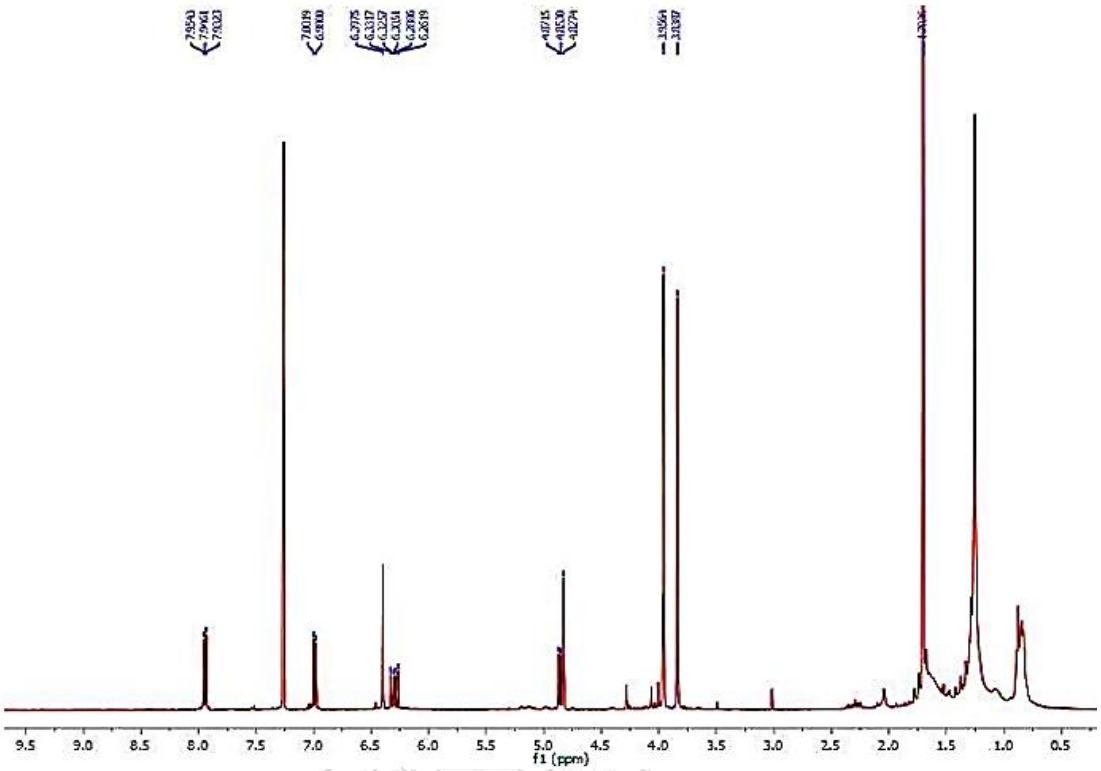

Figure A-1 ^{1}H-NMR spectrum of **1** in $CDCl_3$

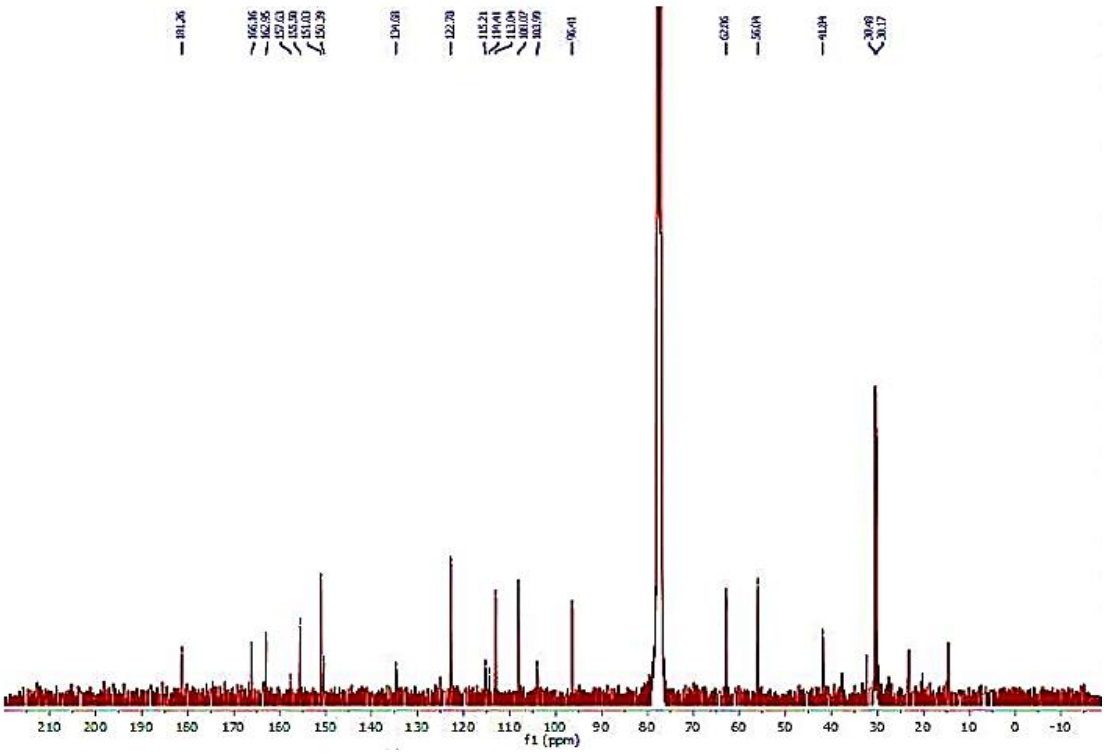

Figure A-2 ^{13}C-NMR spectrum of **1** in $CDCl_3$

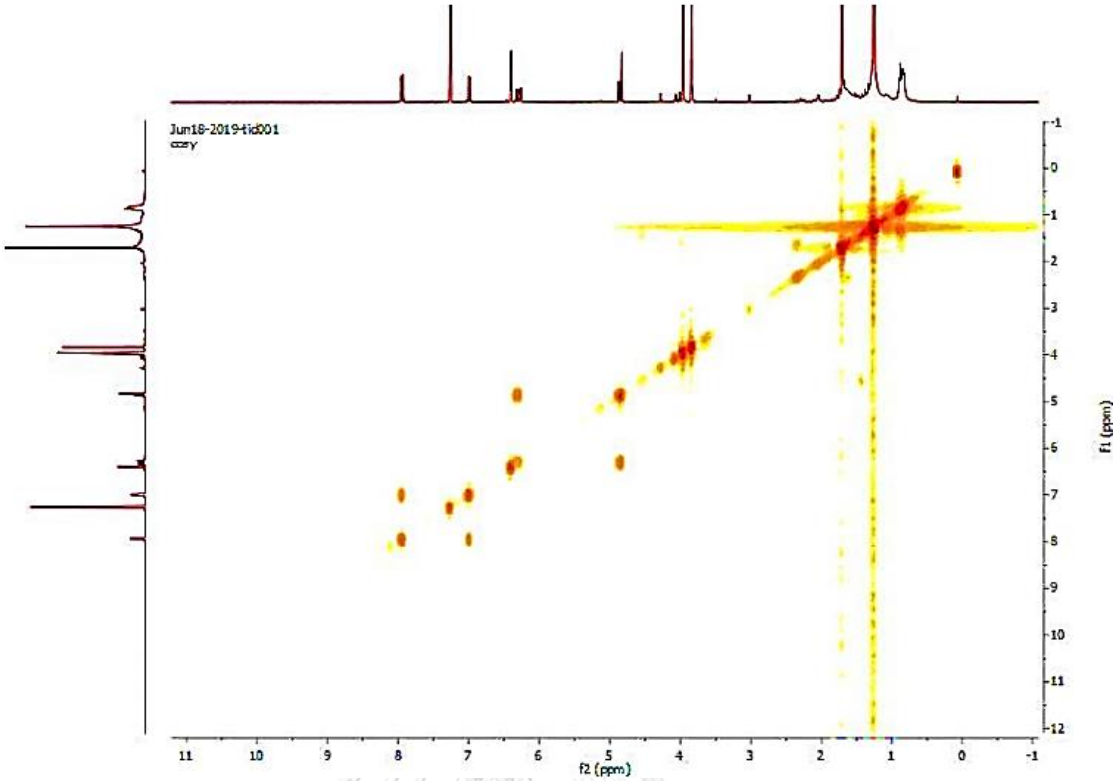

Figure A-3 COSY NMR spectrum of **1** in $CDCl_3$

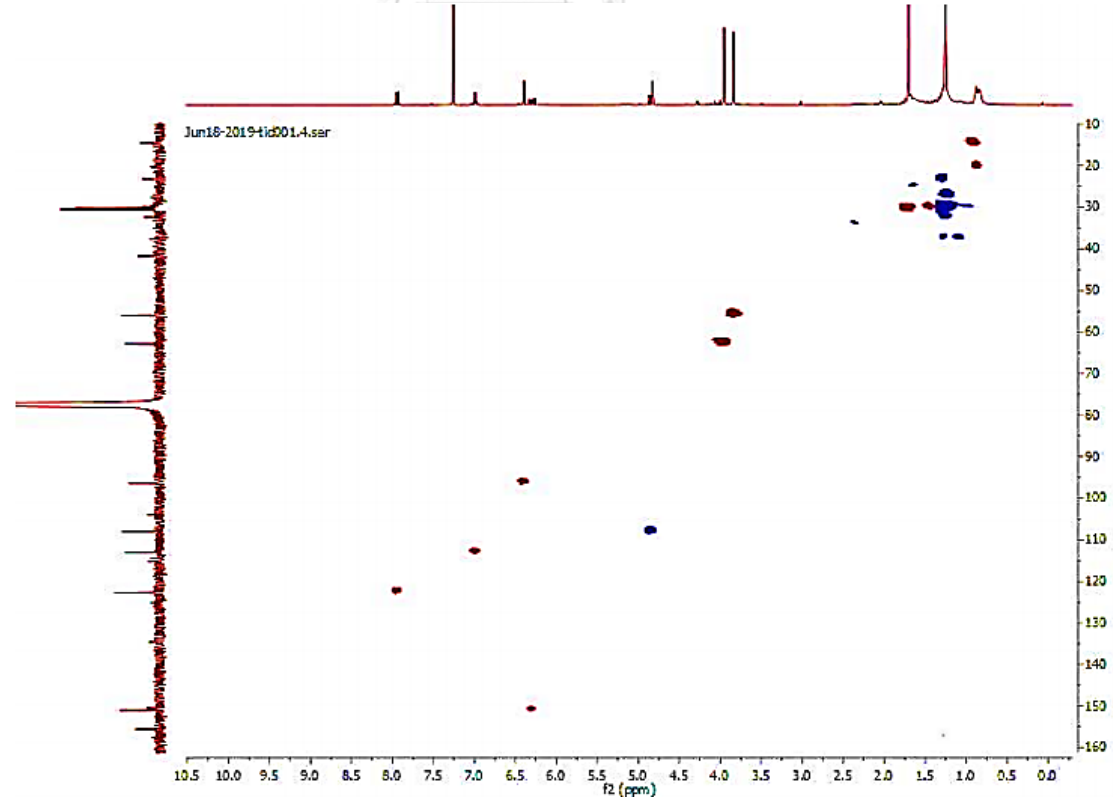

Figure A-4 HSQC NMR spectrum of **1** in $CDCl_3$

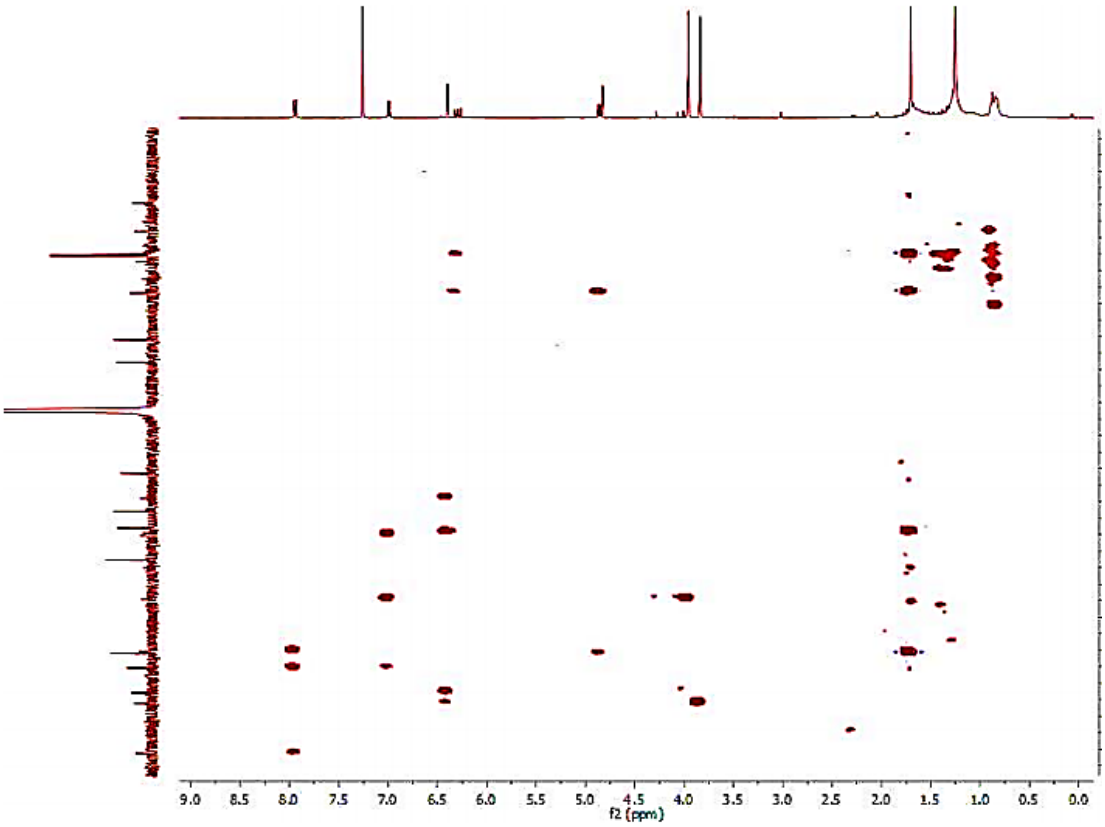

Figure A-5 HMBC NMR spectrum of **1** in $CDCl_3$

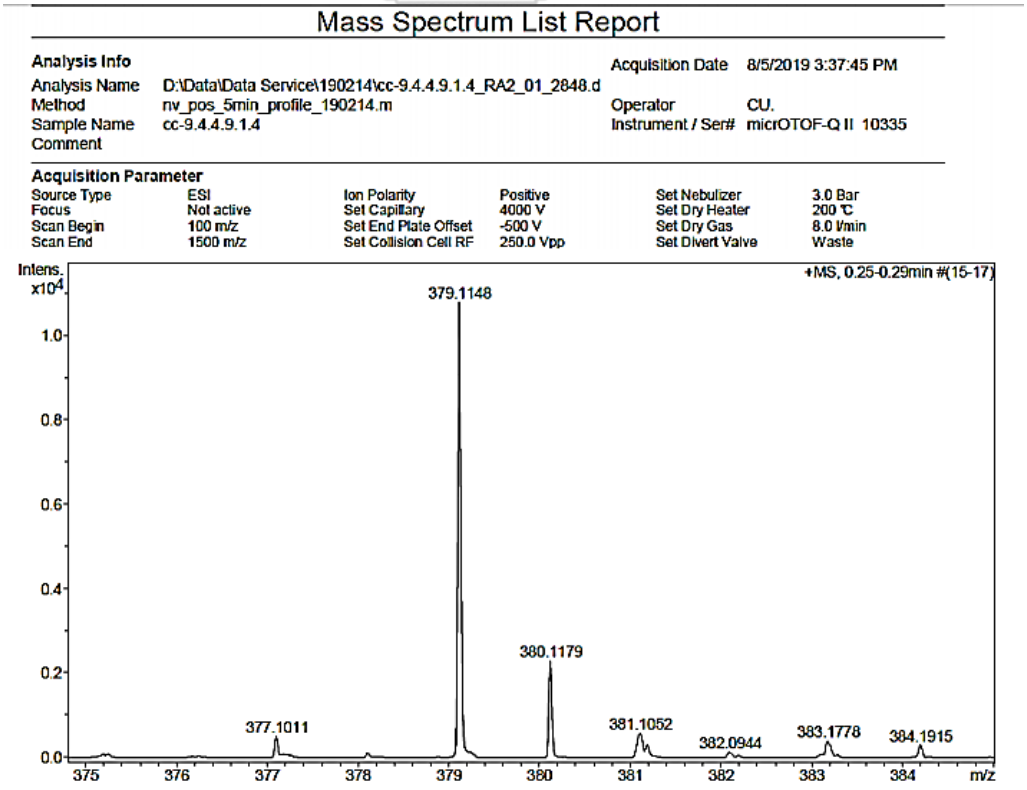

Figure A-6 HRESIMS spectrum of **1** in $CDCl_3$

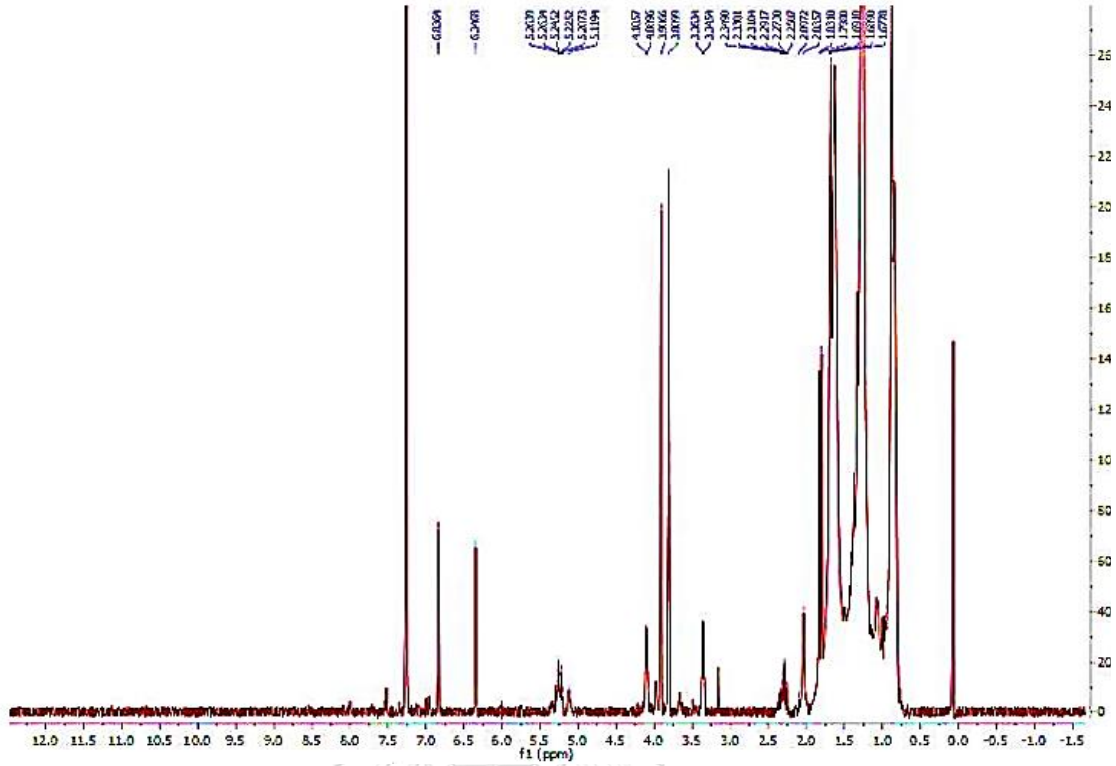

Figure A-7 ^{1}H-NMR spectrum of **2** in $CDCl_3$

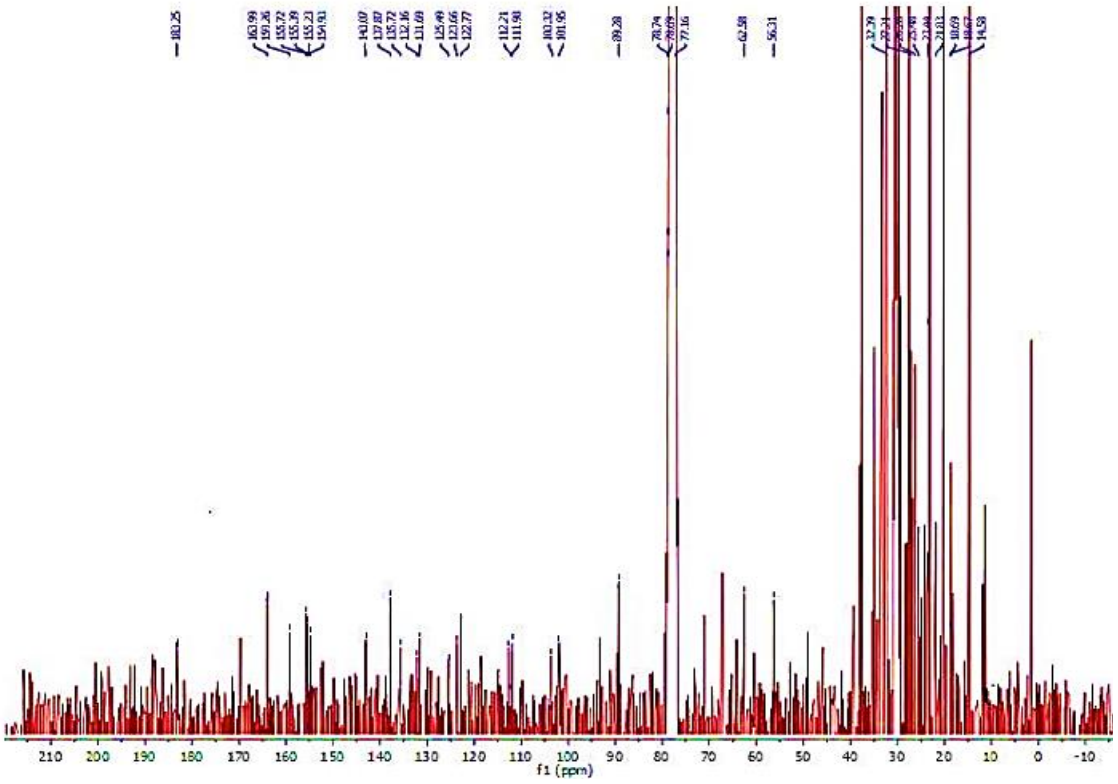

Figure A-8 ^{13}C-NMR spectrum of **2** in $CDCl_3$

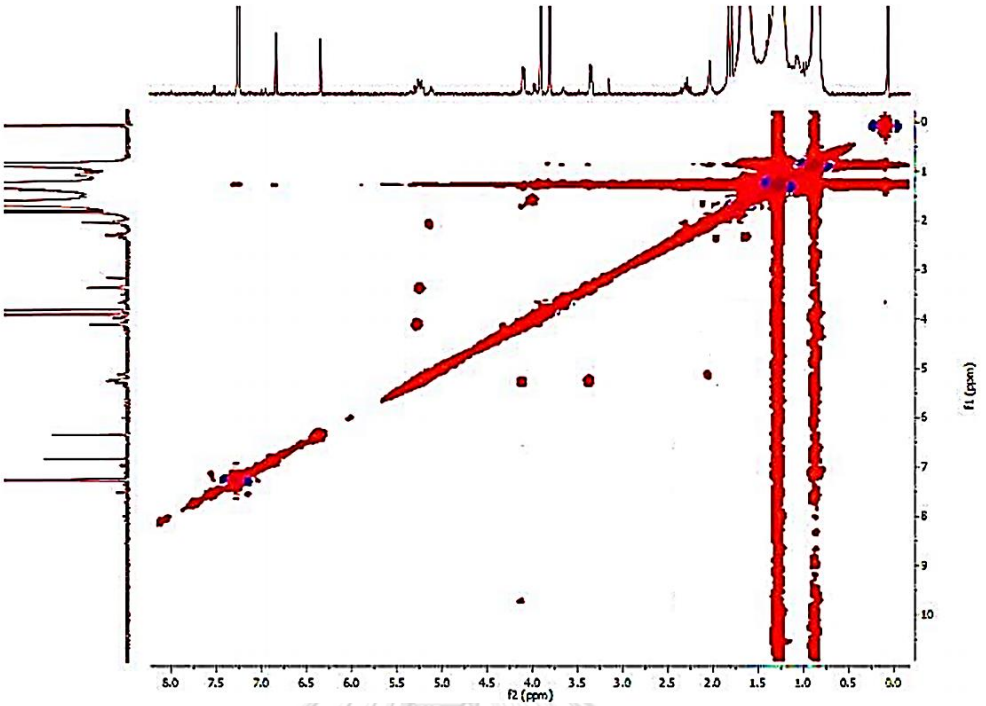

Figure A-9 COSY NMR spectrum of **2** in $CDCl_3$

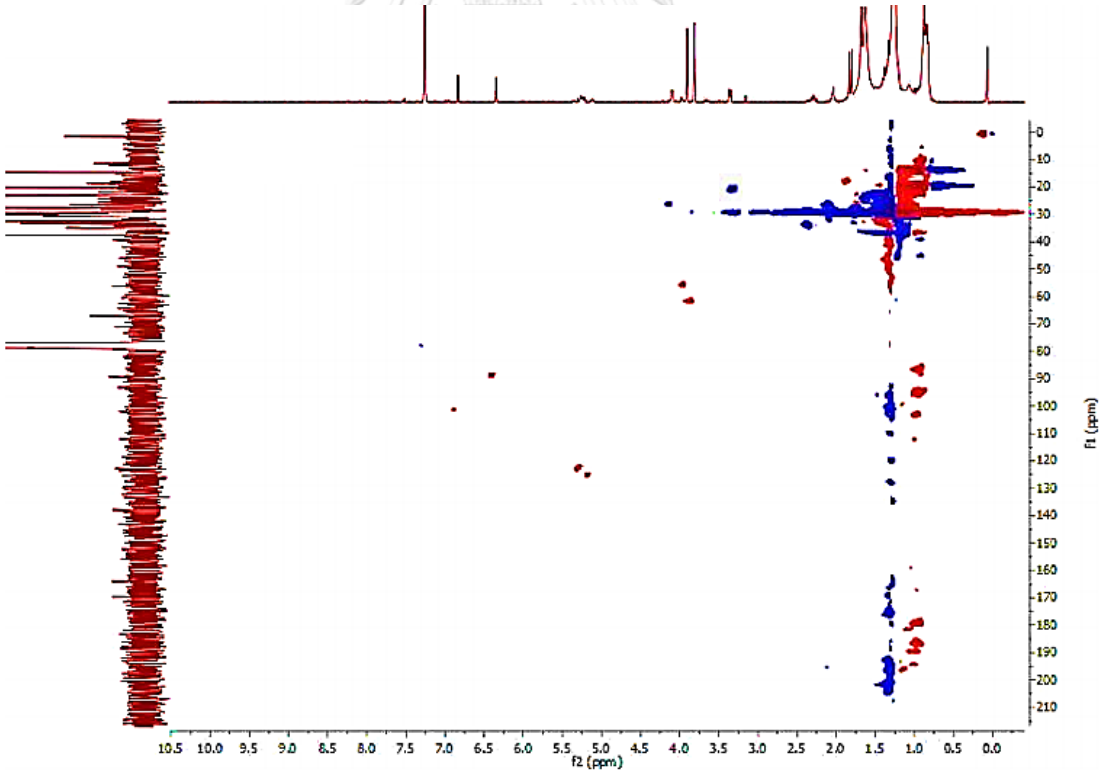

Figure A-10 HSQC NMR spectrum of **2** in $CDCl_3$

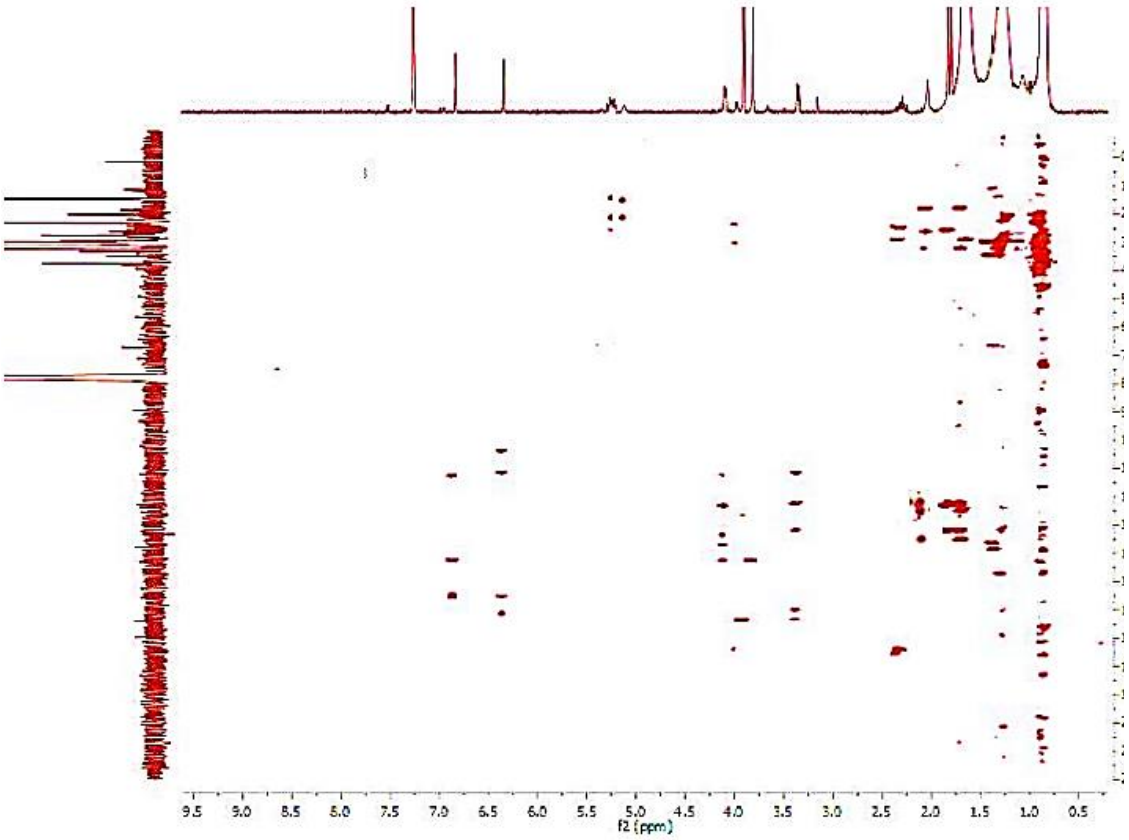

Figure A-11 HMBC NMR spectrum of **2** in $CDCl_3$

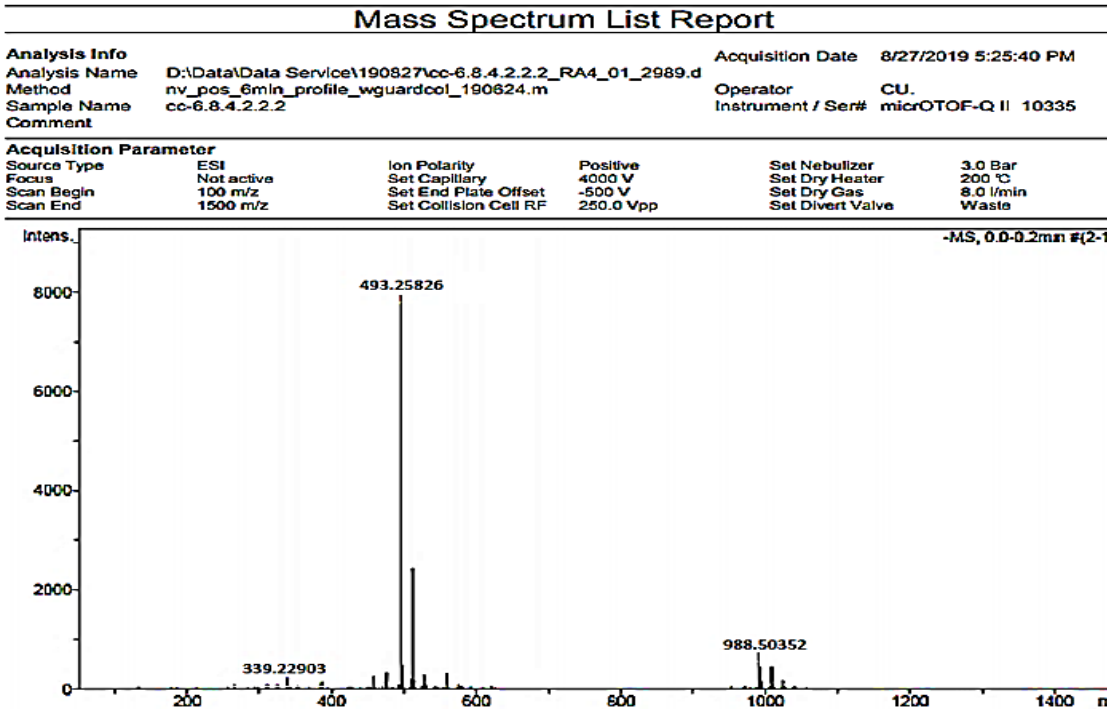

Figure A-12 HRESIMS spectrum of **2** in $CDCl_3$

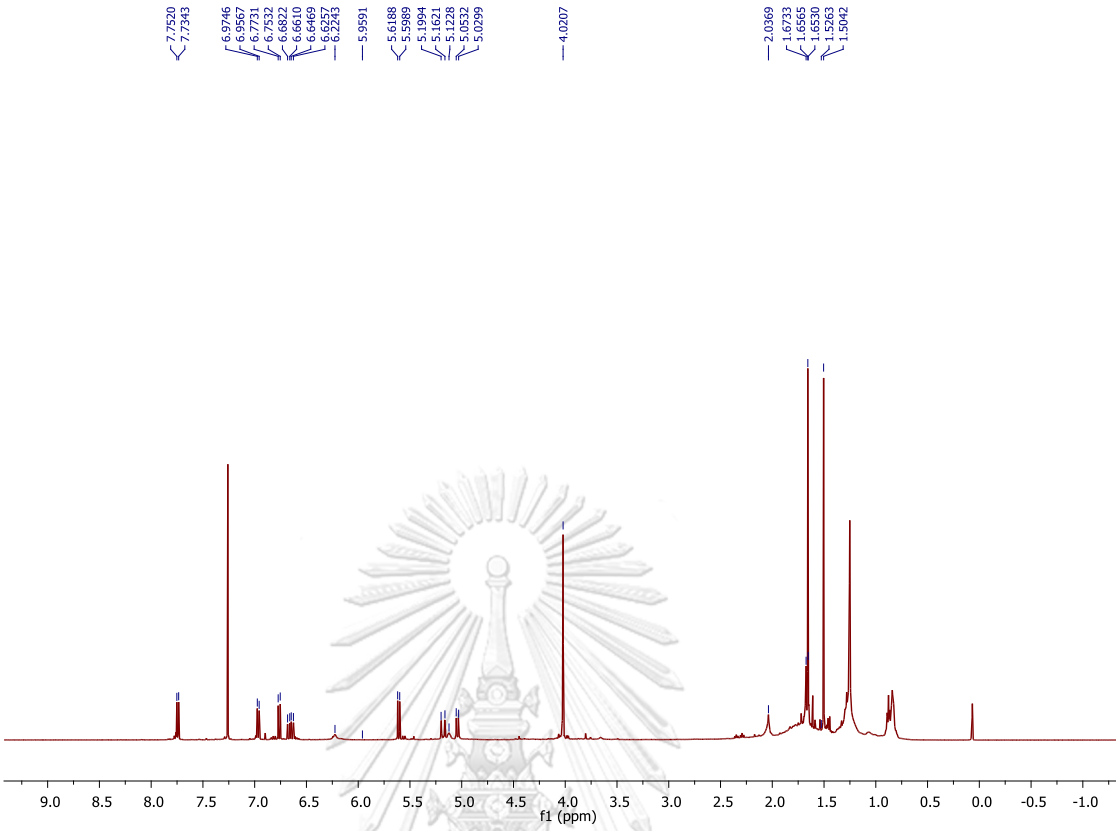

Figure A-13 ^{1}H-NMR spectrum of **3** in $CDCl_3$

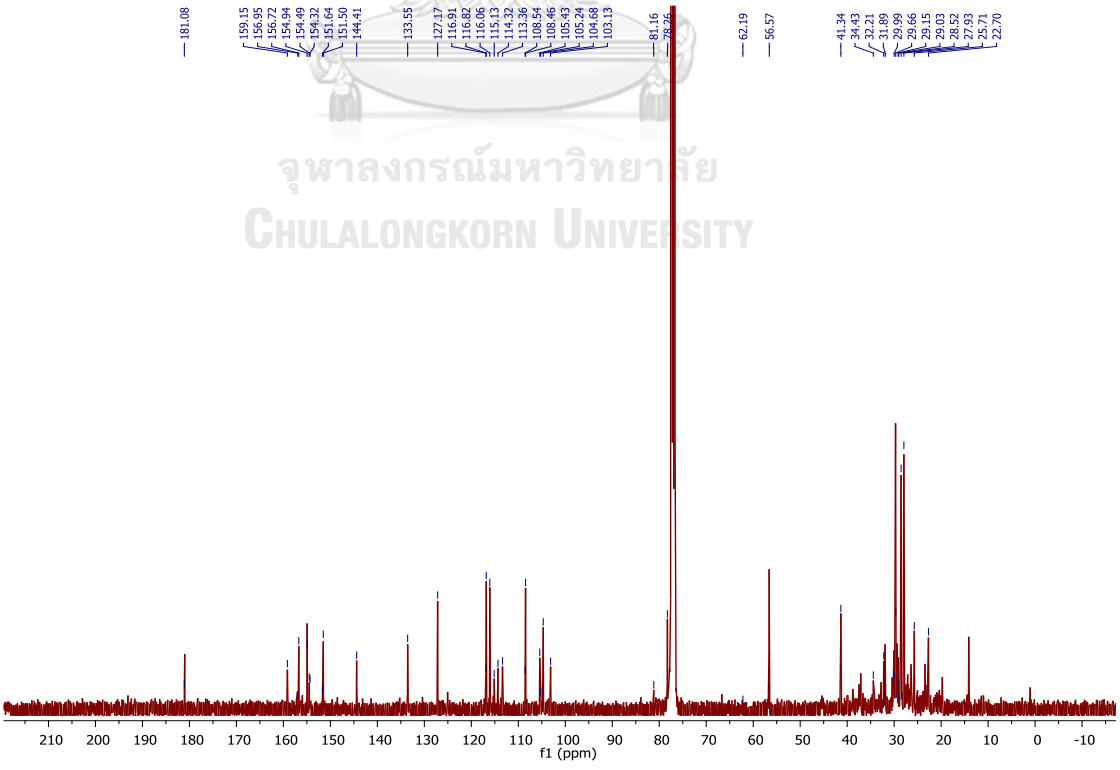

Figure A-14 ^{13}C-NMR spectrum of **3** in $CDCl_3$

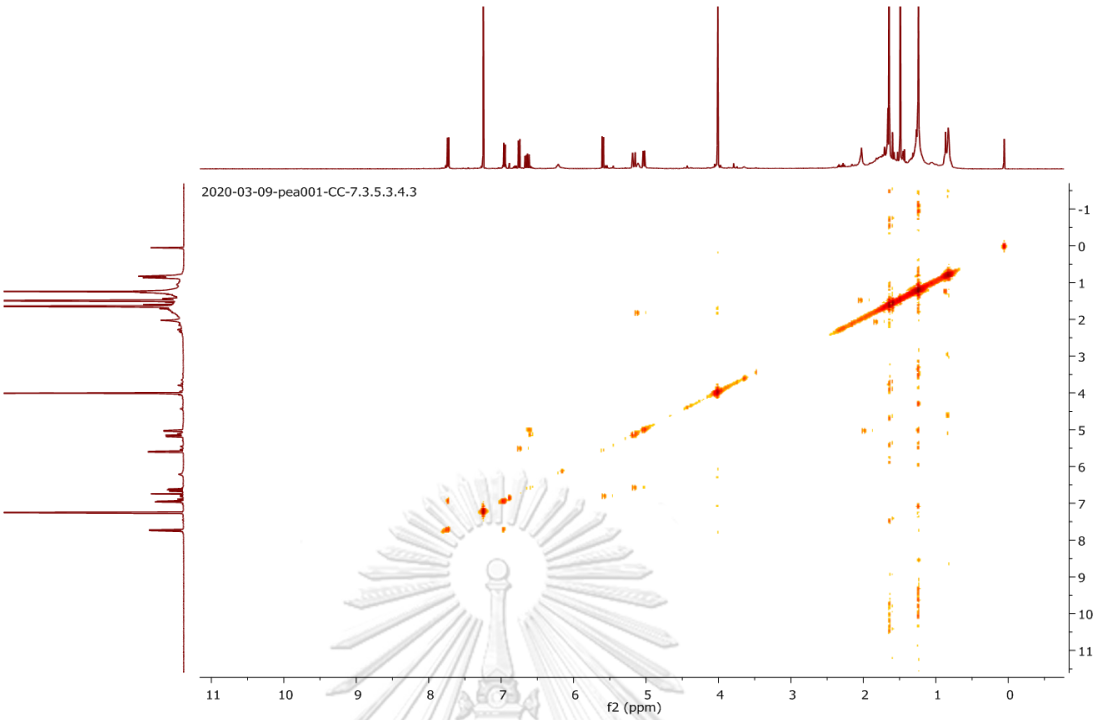

Figure A-15 COSY NMR spectrum of **3** in $CDCl_3$

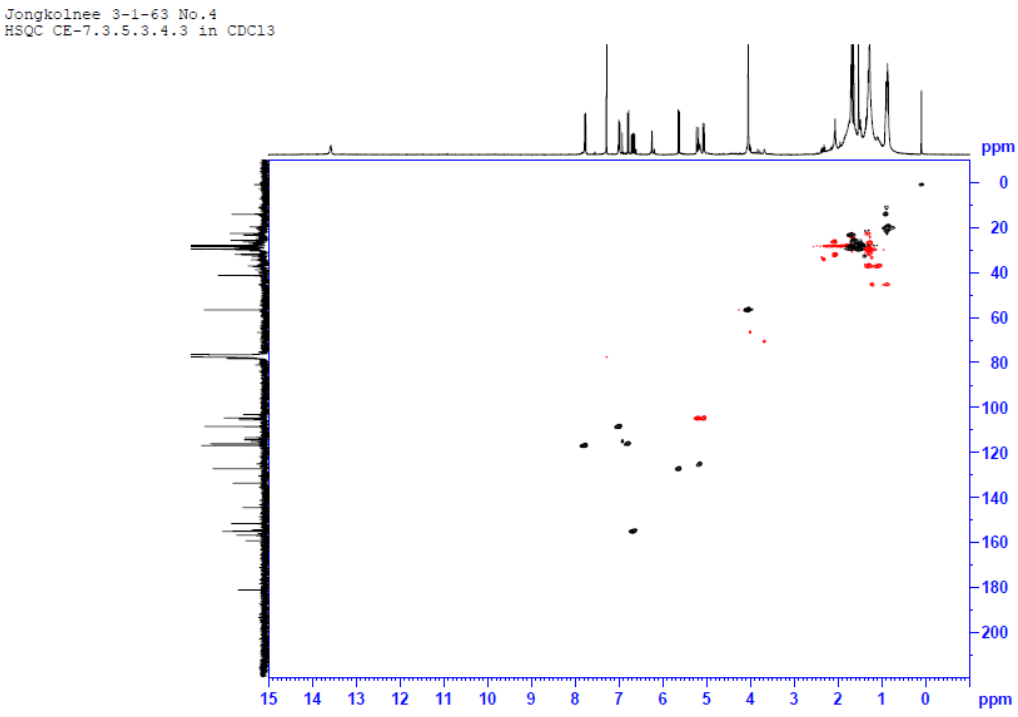

Figure A-16 HSQC NMR spectrum of **3** in $CDCl_3$

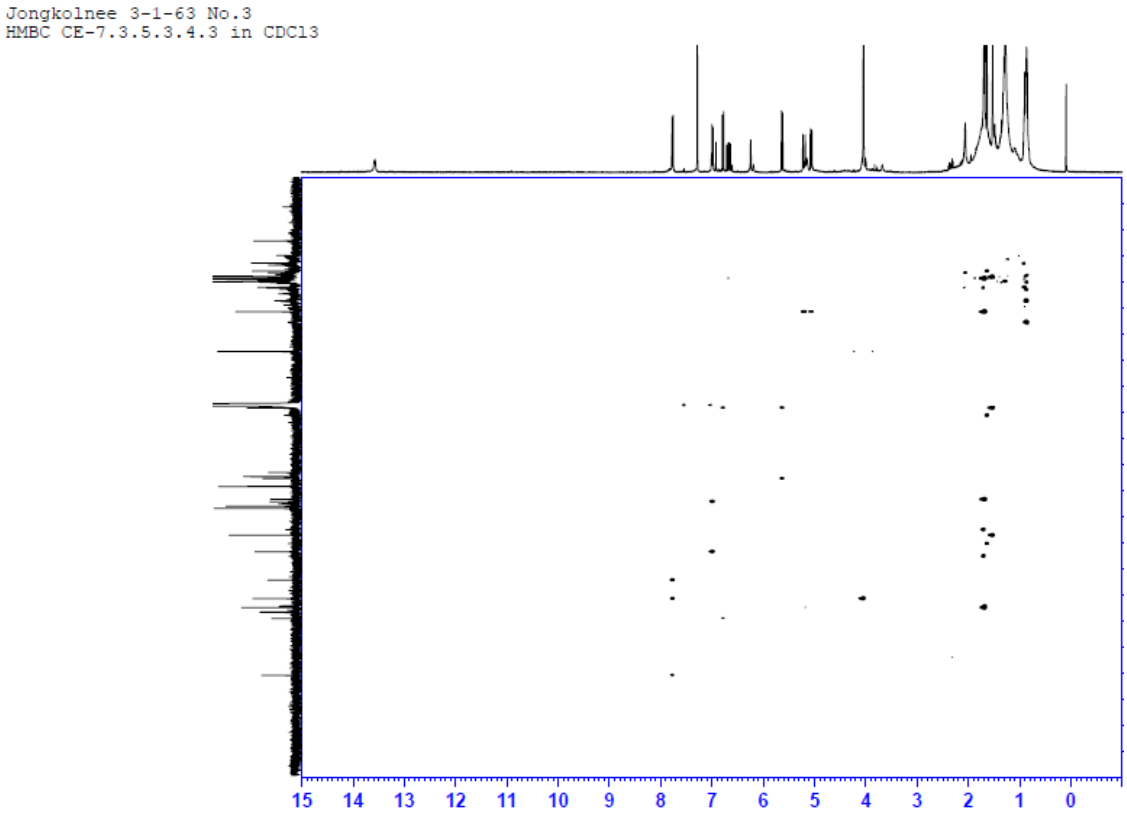

Figure A-17 HMBC NMR spectrum of **3** in $CDCl_3$

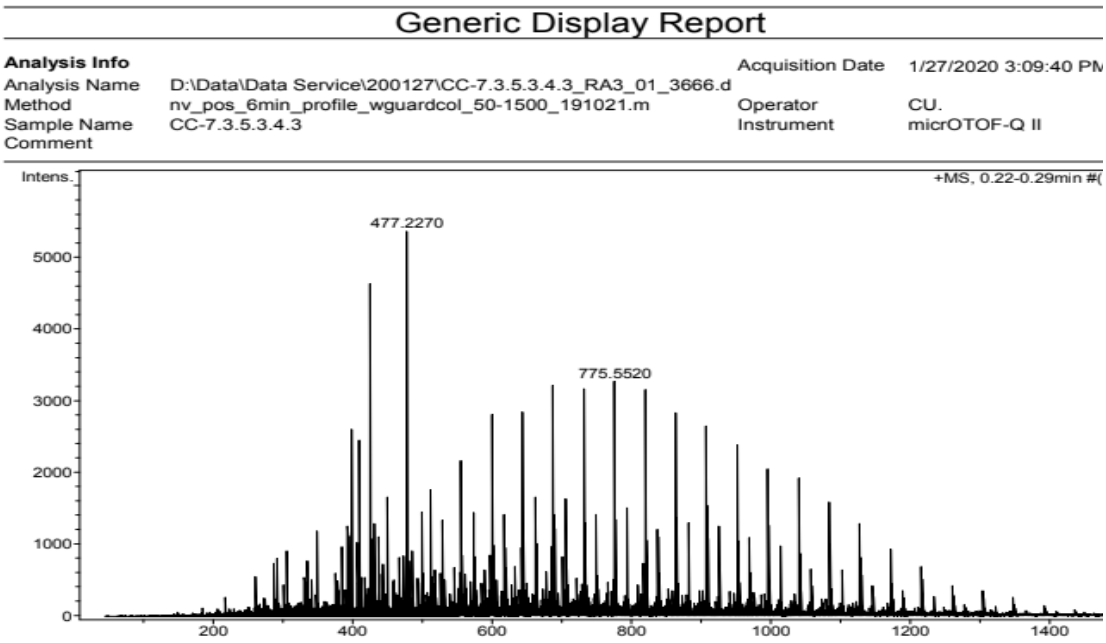

Figure A-19 HRESIMS spectrum of **3** in $CDCl_3$

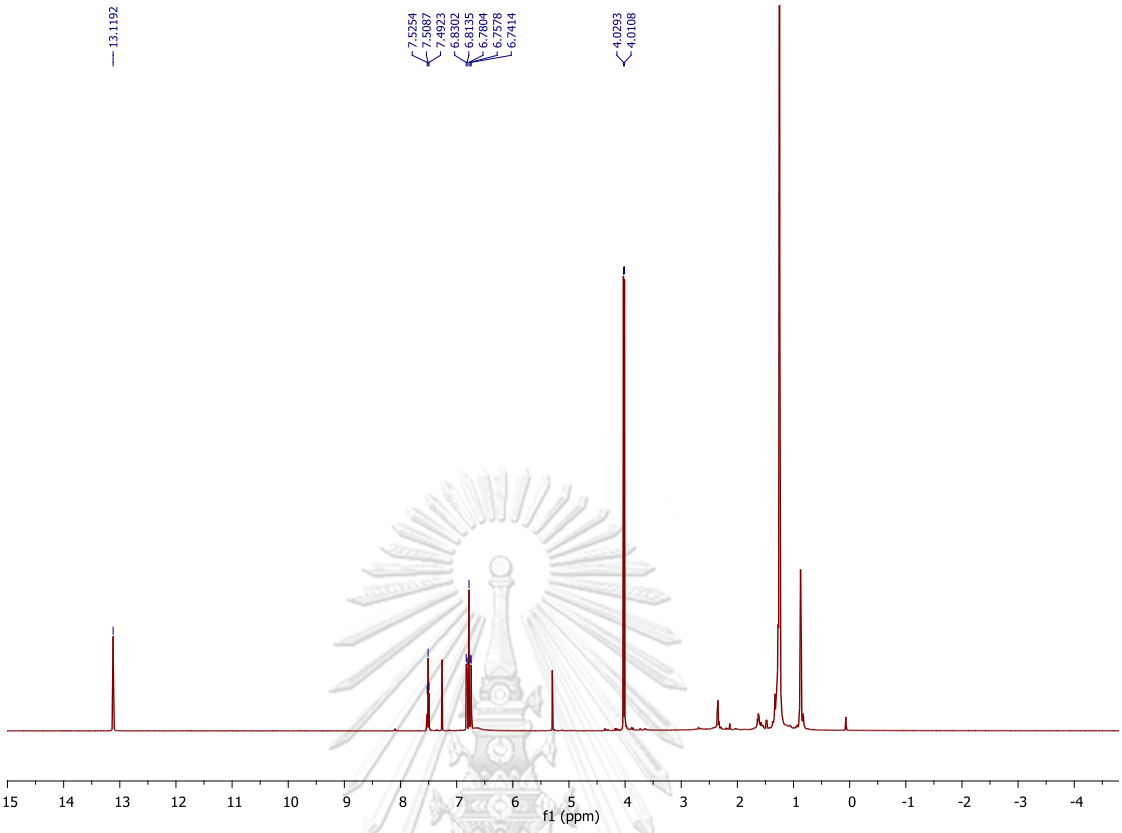

Figure A-19 ^{1}H-NMR spectrum of **4** in $CDCl_3$

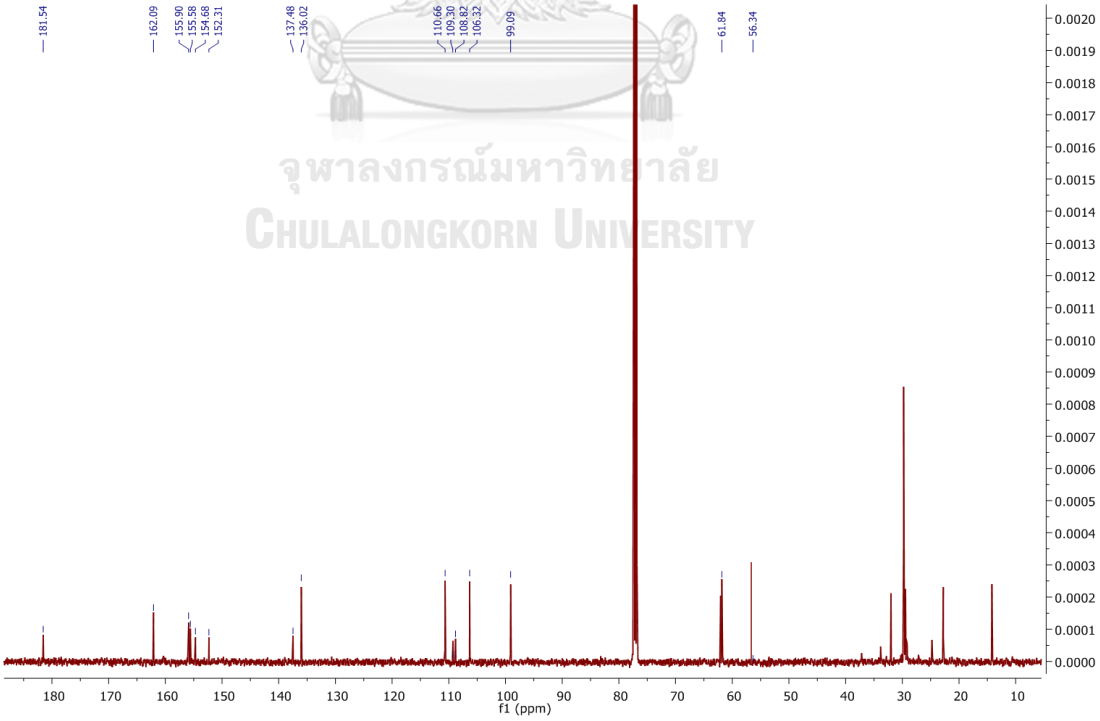

Figure A-20 ^{13}C-NMR spectrum of **4** in $CDCl_3$

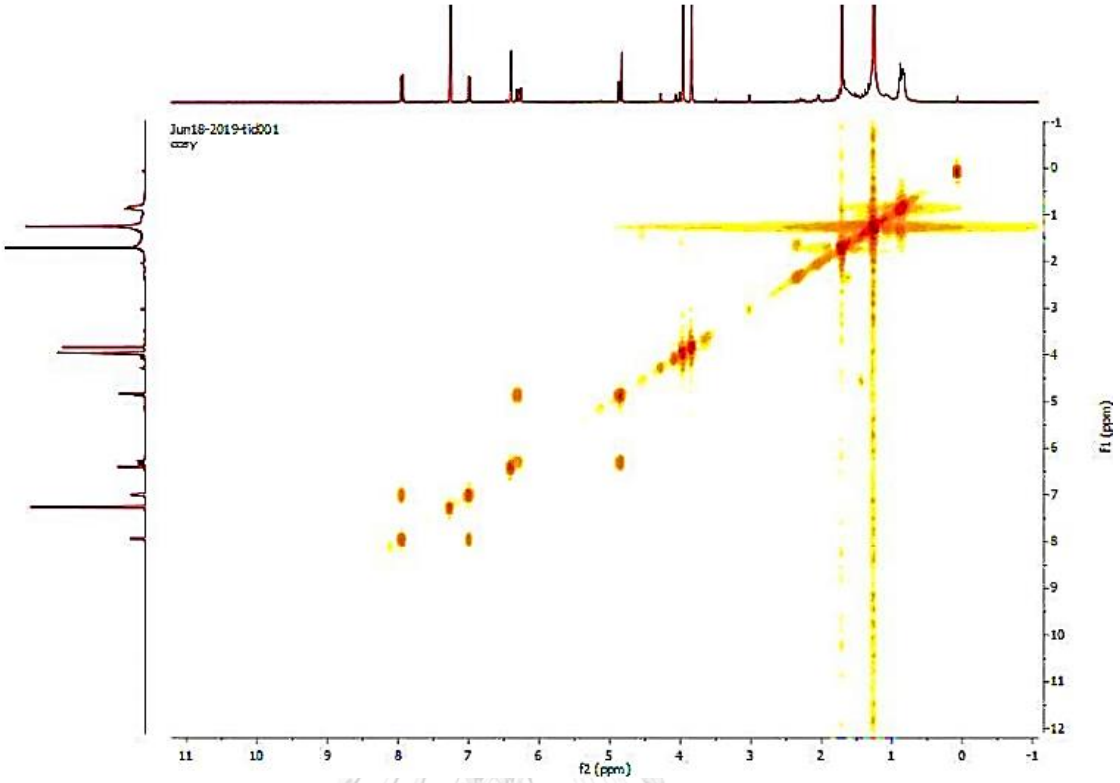

Figure A-21 COSY NMR spectrum of **4** in $CDCl_3$

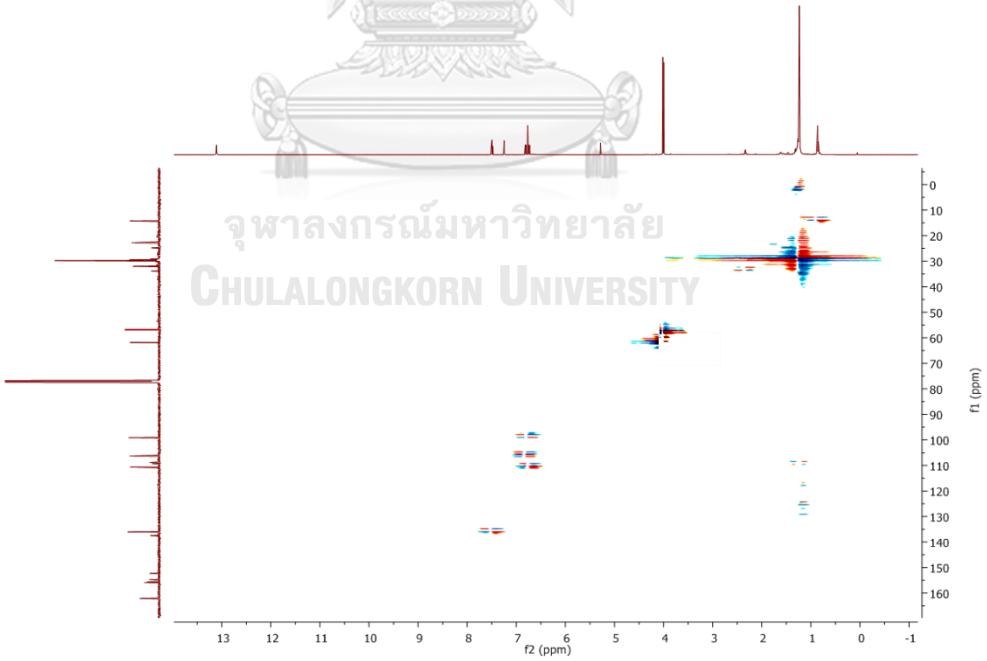

Figure A-22 HSQC NMR spectrum of **4** in $CDCl_3$

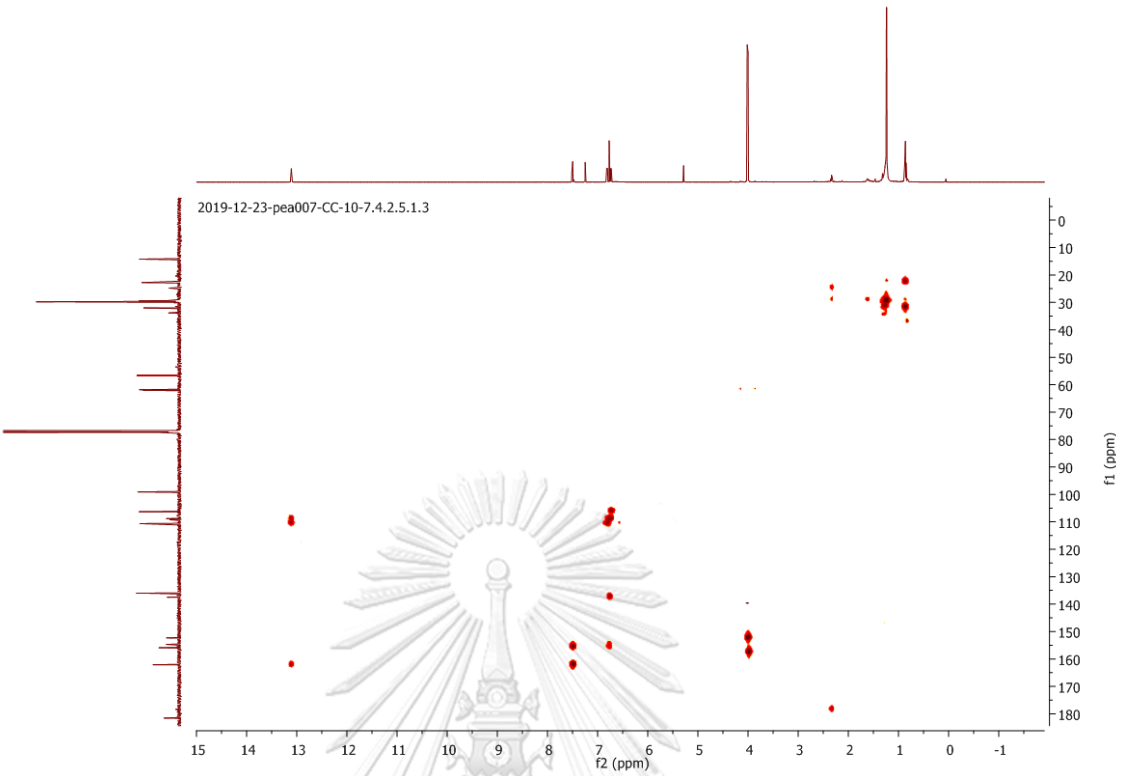

Figure A-23 HMBC NMR spectrum of **4** in $CDCl_3$

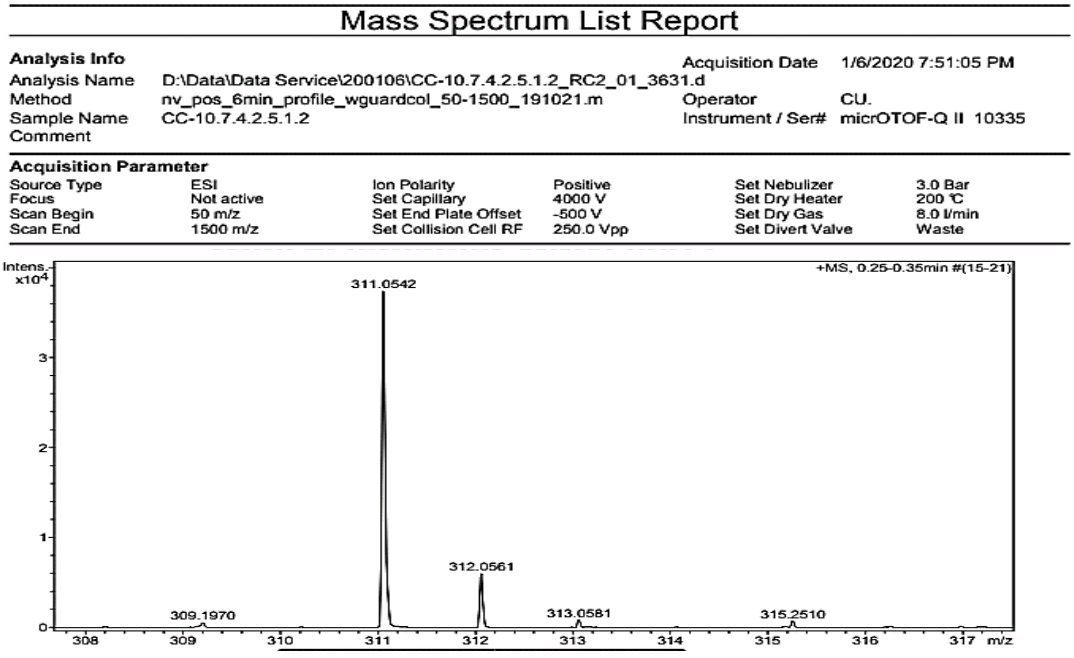

Figure A-24 HRESIMS spectrum of **4** in $CDCl_3$

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